Vol. 33 No. 172 December, 1958

ARCHIVES OF DISEASE IN CHILDHOOD

EDITORS
I. A. B. CATHIE and RICHARD H. DOBBS

THE JOHN CREHAN LIBRARY
JAN 8 - 1959

CONTENTS

PAGE

The Fluid Shift from the Vascular Compartment Immediately after Birth. DOUGLAS GAIRDNER, JOHN MARKS, JANET D. ROSCOE and R. O. BRETTELL 489

The Plasma Amino Acids in Malnutrition: Preliminary Observations. ROLAND G. WESTALL, ELLEN ROITMAN, CARMEN DE LA PEÑA, HOWARD RASMUSSEN, JOAQUIN CRAVIOTO, FEDERICO GOMEZ and L. EMMETT HOLT, Jr. 499

Hypocalcaemic Tetany Developing within 36 Hours of Birth. W. S. CRAIG and M. F. G. BUCHANAN 505

Cellular Immunity at Birth: The Mechanism and Nature of the Phagocytic Response. M. S. EDWARDS, L. L. GRIFFITHS and P. N. SWIFT 512

Thrombotic Microangiopathic Haemolytic Anaemia. M. J. ROBINSON, N. P. ORCHARD and A. R. EDEN 520

Acute Acquired Haemolytic Anaemia Associated with Herpes Simplex Infection. R. MCLAREN TODD and N. V. O'DONOHOE 524

Haemolytic Anaemia in a Child Receiving Nitrofurantoin. DAVID LEVY and ALICE PORGESS 527

Erythrocyte Sedimentation Rate, C-Reactive Protein Determination, Serum Protein Fractions, Fibrinogen Level and Antistreptolysin Reaction in 52 Rheumatic Children. N. POPOV and S. STANISHEVA 529

The Prognosis of Idiopathic Renal Acidosis in Infancy with Observations on Urine Acidification and Ammonia Production in Children. ELIZABETH U. BUCHANAN and G. M. KOMROWER .. 532

Diastematomyelia. C. C. MICHAEL JAMES and L. P. LASSMAN 536

A Case of Cushing's Syndrome in an Infant. E. GOLDBLATT and A. H. SNAITH 540

Epidemics of Gastro-enteritis Associated with *Esch. coli* O119 Infection. K. HUGH-JONES and G. I. M. ROSS 543

Book Reviews 552

Index to Volume 33 553

LONDON
BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, W.C.1

YEARLY SUBSCRIPTION (6 NUMBERS) £4 4s. 0d. U.S.A. $13.50 SINGLE NUMBER 17s. 6d.

EDITORIAL COMMITTEE

F. M. B. Allen
J. J. Mason Brown
P. R. Evans
Isabella Forshall
Douglas Gairdner
John Hay, Jr.
A. V. Neale
Clifford Parsons
K. H. Tallerman
Winifred Young

President of the British Paediatric Association

Editor, *British Medical Journal*

Appointed by the British Paediatric Association and the British Medical Association

GENERAL ADVISORY BOARD

Cecile H. D. Asher (London).
Alan Brown (Toronto, Canada).
N. B. Capon (Liverpool).
Robert Cruickshank (Edinburgh).
Lorimer Dods (Sydney, Australia).
Harry Ebbs (Toronto, Canada).
William Emdin (Cape Town, South Africa).
Jean Mackintosh (Birmingham).
Alan Ross (Montreal, Canada).
H. J. Seddon (London).
Eric Sims (North Adelaide, Australia).
H. L. Wallace (Durban, South Africa).
Howard Williams (Melbourne, Australia).

NOTICE TO CONTRIBUTORS

Papers for publication should be sent to the Editors, B.M.A. House, Tavistock Square, London, W.C.1.

Submission of a paper for publication will be held to imply that it contains original work which has not been previously published. Permission to republish must be obtained from the Editors.

Papers should be as concise as possible and illustrations kept to the minimum. Manuscripts should be typewritten top copies in double spacing. They should have been carefully revised, and alterations in proof, apart from printers' errors, are not permissible.

Photographs and photomicrographs should be submitted unmounted. Drawings and diagrams should be boldly drawn in black ink on stout white paper. Any necessary lettering may be lightly inserted in pencil.

Overseas contributors should nominate somebody resident in Great Britain willing to correct their proofs and deal with the subject of reprints.

Current numbers of the *Archives of Disease in Childhood* should be consulted for information concerning standard abbreviations, etc. In references to papers in the text the year of publication in parenthesis should follow the author's name. References at the end of the text should be in alphabetical order of authors' names, and titles of journals should be abbreviated according to the *World List of Scientific Periodicals*. Book titles should be followed by the place of publication.

Failure to observe the above directions may result in the return of typescripts to authors for correction, with consequent delay in publication.

Twenty-five reprints are supplied free. Further reprints must be ordered when returning proofs.

Subscription. £4 4s. 0d. per annum, post free.

Applications for advertising space should be made to the Advertisement Manager, British Medical Association, B.M.A. House, Tavistock Square, London, W.C.1.

Towards meeting the demands of growth

THE higher metabolic needs of the young which accompany natural growth and development, and which are further increased by heavy daily expenditure of energy, tend to diminish reserves of physical and nervous vitality.

'Ovaltine' provides a useful supplement to help to satisfy the extra needs for proximate and accessory food principles. The malt, milk, cocoa, soya and eggs which 'Ovaltine' contains are blended to form a highly nutritious food beverage —deliciously flavoured and easily assimilated.

It is of value in helping to maintain normal weight increase and is of undoubted help to those recovering from the debilitating effects of the common fevers of childhood. For growing children 'Ovaltine' is a dietary reinforcement which can be routinely recommended with confidence.

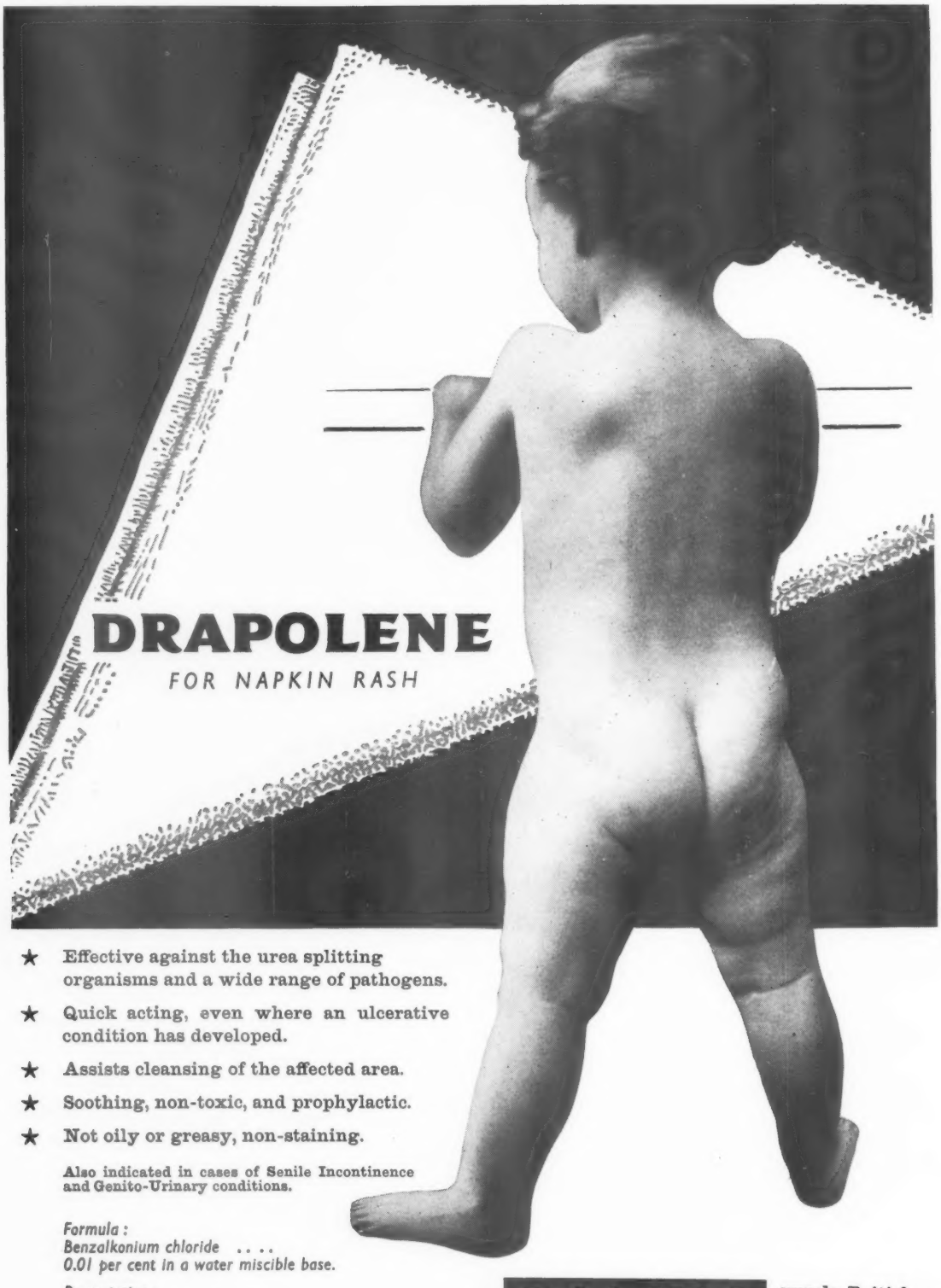
OVALTINE

Vitamin Standardization per oz.—
Vitamin B_1, 0.3 mg.;
Vitamin D, 350 i.u.; Niacin, 2 mg.

A. WANDER LIMITED, 42 UPPER GROSVENOR STREET, LONDON W.1

Manufactory, Farms and 'Ovaltine' Research Laboratories: King's Langley, Herts.

M.384



DRAPOLENE

FOR NAPKIN RASH

★ Effective against the urea splitting organisms and a wide range of pathogens.

★ Quick acting, even where an ulcerative condition has developed.

★ Assists cleansing of the affected area.

★ Soothing, non-toxic, and prophylactic.

★ Not oily or greasy, non-staining.

Also indicated in cases of Senile Incontinence and Genito-Urinary conditions.

Formula :
Benzalkonium chloride . . . .
0.01 per cent in a water miscible base.

Presentation :
2 oz. tubes and 1 lb. jars.
Basic N.H.S. Cost : 2 oz. tube 3/- (exempt P.T.)

CALMIC purely British Pharmaceuticals

CALMIC LIMITED, Crewe, Cheshire. Crewe 3251/7 • London, 2 Mansfield Street, W.1. Langham 8038/9

UNIQUE

among oral antibiotics

BIOTEXIN

TRADE MARK

PROMPT

Biotexin promptly produces bactericidal blood levels far in excess of those achieved by other oral antibiotics

PERSISTENT

Biotexin maintains high antibacterial concentrations in the blood longer than other oral antibiotics

With any other oral antibiotic, divided doses must be given frequently to maintain continuous adequate effect, whatever the total daily dose decided upon. With Biotexin, since it is eliminated very slowly, increase of dose gives a substantial increase in blood level and duration of therapeutic effect. There is actually an advantage in giving fewer and larger doses at longer intervals; 1 gram once a day or 0.5 gram twice daily is usually favoured.

BIOTEXIN

TRADE MARK

novobiocin Glaxo

The **prompt** and **persistent** oral antibiotic in two easy-to-take forms:

125 mg. tablets novobiocin (as sodium salt). Bottles of 32 and 100.

NEW **Syrup** containing 125 mg. novobiocin (as calcium salt), in 5 ml. 2 fl. oz. bottle (12 x 5 ml doses).

GLAXO LABORATORIES LTD., GREENFORD, MIDDLESEX. BYRon 3434

Subsidiary Companies or Agents in most countries

When pancreatin replacement

therapy is indicated, as in

FIBROCYSTIC DISEASE OF THE PANCREAS

prescribe

PANCREATIN GRANULES

TRIPLE STRENGTH ENTERIC-COATED

(CLAY & ABRAHAM)

PANCREATIN B.P.

There are several substances which are used alone or in combination for the enteric coating of pharmaceutical preparations. In 1948, when these granules were originally prepared, a formula was chosen which clinical results have proved to be eminently successful. (*Recent Advances in Paediatrics*, Churchill, 1954, page 256.) Furthermore, a process was evolved by which not merely each granule but each particle of triple strength pancreatin receives its coating to prevent the destructive action of the gastric acid secretion while allowing the enzymes to become active in the duodenum.

Bottles containing eight ounces

LITERATURE ON REQUEST

Also PANFIB—Trade Mark—one grain tablets of pancreatin 5 times B.P. '58 Strength. Bottles of 100 and 1,000

Registered CANDA Trade Mark

CLAY & ABRAHAM (Mnfg.) LTD

Established 1813

UPPER DUKE STREET, LIVERPOOL 1

For happier, healthier motherhood

One factor which has contributed to the encouraging decrease of infant and maternal mortality in civilised countries is the development of modern nutritional knowledge. A preparation which is contributing to healthier and safer motherhood in this country is PREGNAVITE. This comprehensive vitamin-mineral supplement is specifically formulated to fulfil the increased daily requirements of the pregnant or lactating woman.

Packs of 60, 120 and 1,000.
Basic price to N.H.S. 1,000 tablets 32/9

FORMULA *2 tablets, one of each colour, three times a day provide:*

liquid vitamin A conc., B.P. (40 mg.)	2,000 i.u.
liquid vitamin D conc., B.P. (30 mg.)	300 i.u.
vitamin B_1, B.P.	0.6 mg.
vitamin C, B.P.	20 mg.
tocoph. acet., B.P.C. (vitamin E)	1 mg.
nicotinamide B.P.	25 mg.
ferr. sulph. exsic., B.P.	204 mg.
calcium and phosphorous equivalent to 480 mg. calc. phosph., B.P.	
pot. iod., B.P.	not less than 15 p.p.m.
cupr. sulph., B.P. / mang. sulph., B.P.C.	not less than 40 p.p.m.

VITAVEL SYRUP
for children
The delicious flavour of Vitavel Syrup solves the problem of ensuring continuity of treatment where supplementation is indicated

Basic Price to N.H.S.
6 fl. oz. 2/6, 40 fl. oz. 16/-.

BEMAX *for all ages*
Bemax is stabilized wheat germ — the richest natural vitamin-protein-mineral supplement.

Standard Reference Cards
Factual information on all our products is available. Produced in accordance with A.B.P.I. Specifications, cards can be obtained from our representatives or from Vitamins, Ltd.

(DEPT. B.B.7), UPPER MALL, LONDON, W.6. VITAMINS LIMITED

For the prompt
reduction of
Fever
and relief of
pain in children

NEW
PAEDIATRIC
ANTIPYRETIC
ANALGESIC

'ENERIL'
Trade Mark
brand of Paracetamol Elixir

'ENERIL,' a new paediatric elixir, is a stable preparation suitable for the reduction of fever and pain in children.
'ENERIL' provides accurate effective dosage, in pleasantly-flavoured form readily acceptable by children. It does not produce gastric irritation.
Indicated in all conditions where antipyresis and analgesia are required.
There are no contraindications to 'ENERIL.'
Each teaspoonful (4 ml.) contains:
120 mg. Paracetamol (N-acetyl-p-aminophenol).
Dosage : Infants under 12 months, ½ teaspoonful (2 ml.)
Children 1-4 yrs. ½ to 1 teaspoonful (2-4 ml.)
„ 4-8 yrs. 1 to 2 teaspoonfuls (4-8 ml.)
„ 8-12 yrs. 2 teaspoonfuls (8 ml.)
To be given every 4-6 hours or as directed by the physician.

Ref. Jnl. Amer. med. Assn. 1956, 160, 1219.

Presentation: Bottles of 4 fl. oz. and 40 fl. oz. (Dispensing Pack).
Basic N.H.S. Price:
4-oz. pack, 4/2 ; 40-oz. pack, 30/7
(*exempt from purchase tax*)

Samples and literature on request

A Nicholas Product

Aspro-Nicholas Ltd. *Slough, Bucks, England*
(*Ethical Pharmaceutical Division*)

I N T R O D U C I N G

'VALLERGAN'

trade mark *brand*

TRIMEPRAZINE TARTRATE

Trimeprazine is a phenothiazine derivative pharmacologically intermediate between promethazine and chlorpromazine. Its antihistamine action is greater than that of promethazine, but its actions on the central nervous system resemble those of chlorpromazine; although it has a stronger spasmolytic action and a weaker antiadrenaline action than chlorpromazine.

'Vallergan' is indicated for oral use in the relief of pruritus and for the pre-operative sedation of children. In infantile eczema the children scratch less and sleep better. Children premedicated with 'Vallergan' arrive at the anaesthetic room free from apprehension and induction is facilitated. After operation they recover quickly with a minimum of restlessness and vomiting.

For the antipruritic indications 'Vallergan' is available as tablets of 10 mg. and as a syrup containing 2 mg./ml. An increased strength trimeprazine tartrate syrup containing 6 mg./ml., for pre-anaesthetic medication for children, is available as 'Vallergan' Forte.

AN M&B BRAND MEDICAL PRODUCT

MANUFACTURED BY

MAY & BAKER LTD

Distributors

PHARMACEUTICAL SPECIALITIES (MAY & BAKER) LTD · DAGENHAM

MA5814

For safety in treating

Children's infections

'GANTRISIN'

Trade Mark Brand

SYRUP

A palatable suspension of N^1-acetylsulphafurazole

NO BITTER AFTER-TASTE

Dose :—
0·01 to 0·02 g. per pound body-weight, every four to six hours.

'Gantrisin' Syrup contains the equivalent of 0·5 g. sulphafurazole in 5 ml. (one teaspoonful)

Packings:
in bottles of 100 ml. and 500 ml.

ROCHE PRODUCTS LIMITED 15 Manchester Square, London, W.1

THE FLUID SHIFT FROM THE VASCULAR COMPARTMENT IMMEDIATELY AFTER BIRTH

BY

DOUGLAS GAIRDNER, JOHN MARKS, JANET D. ROSCOE and R. O. BRETTELL
From the Cambridge Maternity Hospital

(RECEIVED FOR PUBLICATION MAY 20, 1958)

In an earlier paper (Gairdner, Marks and Roscoe, 1952) we drew attention to the fact that the haemoglobin (Hb) level during the first day of life is generally much higher than at birth. We questioned the adequacy of the current explanation, that any rise in the Hb level after birth is the result of transfer of placental blood, and suggested that the effect merited study. We now present further observations comparing the composition of cord blood at birth with venous blood taken from a few minutes to a few hours after birth. Hb, packed cell volume, (PCV), plasma protein and plasma sodium have been measured. From these comparisons we conclude that after birth there is usually a shift of fluid, mainly whole plasma, from the circulation. The volume of the plasma shift may be large, amounting to an appreciable fraction of the initial plasma volume, and the effect may consequently prove to be of some clinical importance.

Material

Ninety-two infants were studied, of whom 73 were delivered vaginally and 19 by caesarean section. The mother's permission for blood sampling was obtained. Most of the subjects were normal, but a few of the mothers had toxaemia or diabetes; the results in these were similar to those of the normals and have not been separately analysed.

Methods

The cord was always clamped as soon as conveniently possible, both in vaginal and caesarean deliveries. A blood sample was taken by puncture of the umbilical vein between placenta and clamp. Subsequent specimens were taken from an external jugular, internal jugular or femoral vein, or from the ductus venosus (i.e. the continuation of the umbilical vein). The ductus venosus was used particularly for samples within a few minutes of birth. We have found that the ductus venosus can often be catheterized conveniently by inserting a wide-bore trocar into the terminal part of the umbilical vein in the cord, and threading a polythene catheter through it.

Samples were taken into heparinized tubes. Hb and PCV were estimated as described previously (Marks, Gairdner and Roscoe, 1955), Hb being estimated in duplicate in most cases. Plasma protein was estimated by the biuret method, using 0·2 ml. plasma. Plasma sodium was estimated by flame photometer. These four estimations required about 2·5 ml. of blood.

Results

(i) **Change in Hb Level a Few Hours After Birth.** In 92 infants samples were taken one to eight hours after birth and compared with cord blood. These included 19 caesarean deliveries, all but two of which were elective, and thus performed before the onset of labour. Results are shown in Fig. 1. Twenty-two cases showed a negligible change (less than $\pm$ 5%) in Hb level after birth; in two cases there was a fall exceeding 5%, while the remaining 68 (74% of the sample) showed a rise exceeding 5%. This rise amounted to 6-15% in 24 cases, to 16-25% in 23 cases, and to 26% or over in 21 cases. In five cases the rise exceeded 40%, the most extreme example being that of an infant born with an abnormally low haemoglobin level of 10·5 g./100 ml. which six hours after birth had risen to 17·0 g., a rise of 63%. For the series as a whole the mean Hb levels were 16·6 g./100 ml. (S.D. 1·96) for cord blood and 19·1 g./100 ml. (S.D. 2·36) at one to eight hours, giving a mean postnatal rise of 16%. The figure of 16·6 g. for cord blood Hb is not significantly different from 16·9 g., the value found in our earlier series (Marks *et al.*, 1955).

Since the means of the Hb rise (see Fig. 1) are as great at three hours as later (four to six hours), it seems that when the Hb level rises after birth the effect tends to be complete within three hours of birth, and perhaps in much less than this, judging from the few values available for one and two hours.

It will be seen from Fig. 1 that infants delivered by elective caesarean section showed the same order

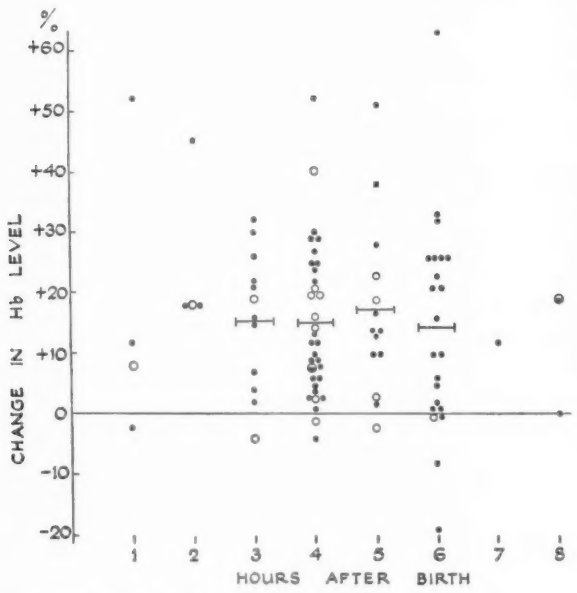


FIG. 1.—Change in Hb level in first few hours after birth.
● 73 infants delivered vaginally.
o 17 infants delivered by elective caesarean section.
◒ 2 infants delivered by non-elective caesarean section.
Averages are shown for 3, 4, 5 and 6 hours.

of postnatal rise in Hb levels as did vaginally delivered infants, a crucial fact which bears on the part played by placental blood transfer in the postnatal Hb rise, to be discussed later.

(ii) **Change in the Hb Level a Few Minutes After Birth.** In order to determine how soon after birth the rise in Hb level begins, in 15 cases one or more venous samples were taken a few minutes after birth, either by means of a catheter introduced into the ductus venosus, or by puncture of another vein. Results are shown in Fig. 2. In seven cases the change in Hb level did not exceed ± 5%; in one case there was a fall of 6·5%; in the remaining seven cases there were rises of from 7% to 19%. It will be noted that in both vaginal and elective caesarean deliveries the Hb level often showed a significant rise within a few minutes of birth. The rises within the first few minutes thus may approach those seen at a few hours and demonstrate that the changes can occur very rapidly after birth.

(iii) **Uniformity of Samples from Different Veins.** At this stage it was felt imperative to establish that the changes were real and did not merely reflect some difference in the composition of blood in different veins. In five infants, the youngest aged 20 minutes, samples were taken from more than one vein. Results (Table 1) show that in no subject did the Hb level vary by more than 0·3 g./100 ml. in different veins, a maximum variation of 1·5%.

(iv) **Change in Packed Cell Volume.** In 39 cases the PCV was estimated in addition to the Hb level,

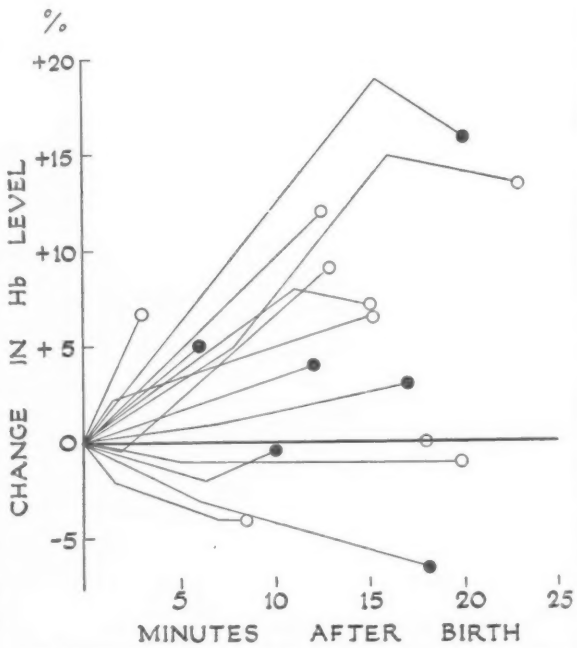


FIG. 2.—Change in Hb level in the first few minutes after birth.
● 6 infants delivered vaginally.
o 9 infants delivered by elective caesarean section.

TABLE 1
UNIFORMITY OF BLOOD SAMPLES FROM DIFFERENT VEINS

Case	Age	Hb (g./100 ml.)				
		Jugular Vein Int.	Jugular Vein Ext.	Femoral vein	Sagittal sinus	Ductus venosus	Difference
1. Normal	20 min.			17·6		17·6	0
2. Mild haemolytic disease	3 hr.	16·3				16·2	0·1
3. Normal	6 hr.	15·2		15·1			0·1
4. Mongol	27 hr.	20·0		20·0	20·2		0·2
5. Mongol	9 days		L. 20·8 R. 20·5		20·5		0·3

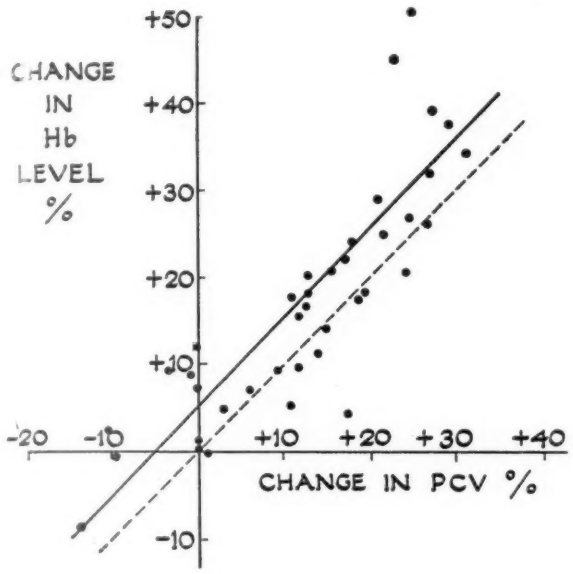


FIG. 3.—Relation of change in Hb level 1-8 hours after birth, to change in PCV, 39 cases. The broken line bisects the axes. Note that the change in Hb tends to exceed the change in PCV

and in Fig. 3 the percentage change in PCV is compared with the percentage change in Hb level. If the Hb and PCV had moved strictly in parallel, the points would have been grouped around the broken line shown; in fact the majority fall well above this line, i.e. the rise in Hb level is usually proportionately greater than the rise in PCV level. The calculated regression line is shown. In order to test the significance of this trend the percentage change in PCV was compared with the percentage change in Hb by the χ^2 test. The difference was highly significant ($\chi^2 = 215{\cdot}9$, d. of f. $= 38$, $p =< 0{\cdot}001$).

Since the ratio $\frac{\text{Hb concentration}}{\text{PCV}}$ defines the mean corpuscular Hb concentration (MCHC), these results are equivalent to stating that after birth there is a significant rise in MCHC. In a series of 21 cases* the mean MCHC of cord blood was 32·3 and in blood at one to eight hours 33·6, an increase of 4·0%.

* It was discovered that one of the centrifuges used had been running at less than the prescribed 3,000 r.p.m. leading to incomplete packing of red cells and to incorrectly high PCV values. Therefore the 18 cases involved were excluded when calculating MCHC values, leaving the 21 cases quoted.

These 18 cases have not been excluded from the data for Fig. 3 for the following reason. Since the effect of incomplete packing is relatively greater in blood samples having a high PCV, the percentage increase in PCV after birth would tend to have been *over*-estimated. The point we have sought to demonstrate in Fig. 3 is that when, after birth, the Hb rises, the PCV tends to rise relatively *less*. The error under consideration would therefore tend to shift results in a direction opposite to that of the trend we are demonstrating.

(v) **Change in Plasma Protein Concentration a Few Hours After Birth.** Plasma protein was measured in 17 cases, care being taken to reject any sample showing haemolysis, to which blood samples in the early days of life are annoyingly prone. The protein concentration showed either a negligible change of less than ± 5% (six cases) or a small postnatal rise of from 6% to 20% (11 cases). The mean value for cord blood was 6·5 g./100 ml. and at one to eight hours was 7·0 g., a mean postnatal rise of 7·7%.

(vi) **Change in Plasma Sodium Concentration.** This was measured in 14 cases. The mean value for cord blood was 145 mEq./l and at one to eight hours was 146 mEq./l, a non-significant difference.

Discussion

To summarize the main findings, within a few hours of birth, there is frequently:

(1) A substantial rise in the Hb level, which reaches its maximum within three hours. In many cases this rise exceeds 25%, and it may reach 50% or more. In some cases the rise exceeds 10% within 15 minutes of birth.

(2) A rise in MCHC, averaging 4·0%.

(3) A rise in plasma protein concentration of up to 20%.

(4) No change in plasma sodium concentration.

Before considering the possible mechanisms responsible for these changes, it is essential to define certain terms used throughout the discussion and to relate them to each other.

In order to estimate the relative proportion of red cells in a sample of blood, we have centrifuged it in a Wintrobe tube for 30 minutes. The proportion of red cells thus determined is the *packed cell volume* (PCV). The PCV does not in fact represent the true volume of the red cells in the blood, since there is a small amount of trapped plasma in the red cell layer. Within the ranges of haematocrit and centrifugation applicable to this study, this can be allowed for by utilizing a factor of 0·95 (Chaplin and Mollison, 1952). The PCV thus corrected is the *venous haematocrit*. Thus,

$$\text{venous haematocrit} = 0{\cdot}95 \times \text{PCV}.$$

Blood in small vessels contains a greater proportion of plasma than does that in large vessels. Consequently the haematocrit of blood from a vessel such as a vein (venous haematocrit) is higher than the true *body haematocrit*. The ratio

$$\frac{\text{body haematocrit}}{\text{venous haematocrit}}$$

has been found to have a value of 0·91 in adults (Chaplin, Mollison and Vetter, 1953) and of 0·87

in newborn infants a few hours old (Mollison, Veall and Cutbush, 1950). Thus for an infant after birth,

$$\text{body haematocrit} = 0{\cdot}87 \text{ venous haematocrit}$$
$$= 0{\cdot}87 \times 0{\cdot}95 \times \text{PCV}$$
$$= 0{\cdot}83 \times \text{PCV}.$$

The postnatal rise in the haemoglobin level might be brought about by one of three possible mechanisms:

(1) A redistribution of plasma within the vascular compartment.

(2) An addition to the red cell volume.

(3) A loss of plasma fluid.

(1) **Redistribution of Plasma Within the Vascular Compartment.** As has already been stated, the ratio $\frac{\text{body haematocrit}}{\text{venous haematocrit}}$ in newborn infants a few hours old has the value of 0·87. In the foetus before birth there is no direct evidence of such a difference between the haematocrit in different parts of the circulation. If we choose to suppose that in the foetus the distribution of red cells and plasma is uniform, after birth a redistribution of plasma would take place, with some of the plasma in effect pooling in the smaller vessels. Then, considering an infant where *at birth* the venous haematocrit is h_1, this will be identical with the body haematocrit, while *after birth* the venous haematocrit h_2 will be given by

$$h_2 = \frac{\text{body haematocrit}}{0{\cdot}87} = \frac{h_1}{0{\cdot}87},$$

from which the percentage rise in haematocrit after birth $100\left(\frac{h_2 - h_1}{h_1}\right)$ can be calculated as 15%.

Since PCV is proportional to venous haematocrit this figure of 15% would also be the percentage increase in PCV after birth.

Under these hypothetical conditions, then, redistribution of plasma within the vascular compartment could in theory cause the PCV to rise by as much as 15%, but not more. However, rises greater than 15% are often observed (see Fig. 3).

While, therefore, we have no reason to believe that in foetal life the small vessels do contain less plasma relative to large vessels than is the case in the infant after birth, we cannot exclude this possibility. But if after birth some redistribution of plasma within the vascular compartment does occur, then it is clear on quantitative grounds that this can do no more than make some contribution to the large rise in PCV which is often observed.

(2) **Addition to the Red Cell Volume.** It will be convenient first to define the approximate size of the increase in red cell volume which would be necessary to produce the observed effect. This can be calculated in terms of the change in PCV for an average infant as follows.

We may take as a typical instance an infant of 3·4 kg., with a PCV at birth of 53%, rising by 15% after birth to 61%, corresponding therefore to a body haematocrit of 44% at birth and 50·6% after birth. With a PCV of 61% the blood volume after birth, obtained from the data of Mollison, Veall and Cutbush (1950) can be estimated to be 90 ml./kg. or 306 ml. If it be assumed that (*a*) the plasma volume remains the same after birth as before birth, then the composition of the circulating blood will be as shown in Fig. 4 (a), while if (*b*) the blood volume be assumed to remain constant, then the state of affairs after birth will be as in Fig. 4 (b).

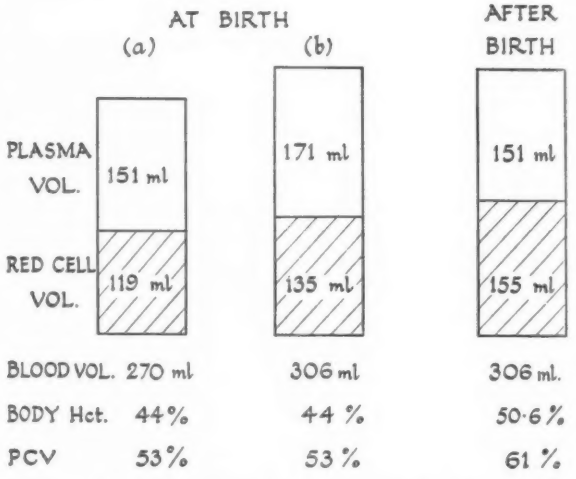


FIG. 4.—Calculated changes in red cell volume after birth, to account for an observed change in PCV from 53% at birth to 61% after birth: (*a*) assuming *plasma* volume unchanged, (*b*) assuming *blood* volume unchanged.

It will be seen that the calculated gain in red cell volume after birth is either (*a*) 155-119 = 36 ml., which is 23% of the initial red cell volume, or (*b*) 155-135 = 20 ml. which is 15% of the initial red cell volume, depending on which of the two assumptions is made.

This calculation gives an idea of the order of magnitude of the gain in red cell volume, i.e. 20 to 36 ml., which would be necessary to account for a postnatal rise of 15% in PCV such as is observed in a typical infant. With these figures in mind we may proceed to consider three possible sources of red cells which might be added to the circulation after birth. (It should be appreciated that many infants show a much greater postnatal rise in PCV

than 15% (see Fig. 3) and would consequently require even larger red cell supplements.)

(*a*) RED CELLS DERIVED FROM TRANSFER OF PLACENTAL BLOOD. In the past it has often been observed that the mean Hb concentration is higher on the first day of life than in cord blood, and where attention has been paid to this difference it has usually been assumed that the postnatal rise in Hb level is the result of placental blood transfer. This view received weighty support from the work of DeMarsh, Windle and Alt (1942, 1948) who compared the Hb and haematocrit of cord blood with that of blood from the sagittal sinus at a few hours after birth. The latter gave Hb and haematocrit values on an average some 12-15% higher, but only if the cord clamping had been delayed, no such difference being seen in infants whose cord had been clamped at once. These authors' findings are thus completely at variance with our own, since throughout our series the cord has been clamped as soon as conveniently possible, yet a significant postnatal rise in Hb level has been observed in the majority of infants.

We have sought without success to find an explanation for this difference in results. Thus DeMarsh and co-workers took their blood samples from the sagittal sinus, a source we have not used in this series. However, a number of our samples were taken from the internal jugular vein, which, as the direct continuation of the sagittal sinus, is most unlikely to contain blood of different composition. Furthermore, reference to Table 1 shows that in two subjects investigated the Hb level of sagittal sinus blood was the same as in other veins.

The possibility exists that significant amounts of placental blood might be rapidly transferred to the baby during the terminal stages of delivery. In order to establish the fact that the postnatal rise in Hb level occurs independently of placental blood transfer, the effect was studied in 17 infants delivered by elective caesarean section, before the onset of labour. In these cases, too, the cord was clamped as soon as possible, so that it seems certain that no appreciable amount of placental blood could have been transferred to the infant. Nevertheless a postnatal rise in Hb level took place in much the same proportion of these subjects as in those delivered vaginally (Fig. 1), and this rise often exceeded 20%.

We must conclude that the postnatal rise in Hb level occurs independently of placental blood transfer (although it is, of course, likely to be augmented by any placental blood transfer that is allowed to take place).

(*b*) RED CELLS DERIVED FROM RESERVOIR SOURCES. The spleen which weighs only about 10 g. at birth is clearly incapable of providing any significant addition to the red cell volume.

The only other viscus which requires consideration is the liver. This highly vascular organ weighs about 150 g. at birth, but even so it seems hardly conceivable that it could be capable of releasing as much as 20 ml. of red cells, or 13% of its weight.

(*c*) NEW RED CELLS DERIVED FROM HAEMOPOIETIC CENTRES. According to Seip (1953) red cells delivered from haemopoietic tissue into the circulation are all reticulated and persist as reticulocytes for about one day. A large addition of new red cells would thus result in a correspondingly large rise in the number of circulating reticulocytes. Therefore, in the present context, where an average rise of the order of 15% in the red cell volume is being considered, the proportion of reticulocytes would be expected to rise from a normal level at birth of 3-4%, to nearly 20%. It may be said at once that no rise of this magnitude occurs. Wegelius (1948) found a slight transient rise in the reticulocyte level from 2·8% at birth to 3·7% at two hours. Seip (1955) also found that the level rose slightly, e.g. from 4·1% at birth to 4·5% after birth, but pointed out that this is what would be expected from the fact that new reticulocytes are added to a smaller volume of blood after elimination of the placental circuit. Our own data (Gairdner *et al.*, 1952) have given similar results, the reticulocytes numbering between 3% and 4% both in cord blood and in blood taken during the first day. These results therefore show that red cells from haemopoietic centres cannot be the source of the postnatal rise in haemoglobin level.

Thus we may conclude that addition to the red cell volume does not play a significant part in the haemoglobin rise that we have observed in infants after early clamping of the umbilical cord.

(3) **Loss of Plasma Fluid.** By exclusion of the other possible mechanisms we must conclude, then, that the postnatal rise in Hb level must be mainly due to haemoconcentration from a shift of fluid from the vascular compartment.

Two other simultaneous changes support the idea of fluid shift:

(i) A rise in the concentration of plasma protein.

(ii) A rise in the MCHC of the red cells.

The increase in the MCHC implies that there is usually some contraction of the red cells from shift of intracellular fluid into the plasma, at the same time as fluid is extruded from the vascular compartment.

If the blood volume after birth is v, the red cell

and plasma volumes at birth, and a few hours after birth, can be defined in terms of the Hb level and the PCV *at birth* (Hb_1, PCV_1) and *after birth* (Hb_2, PCV_2) as shown in Fig. 5.

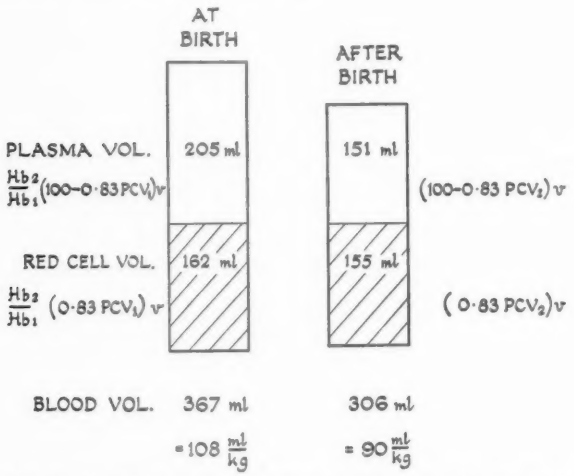


FIG. 5.—Method of calculating red cell and plasma volumes at birth and after birth, in terms of blood volume after birth (v). Numerical values for a typical infant (see text) are shown.

Using this general scheme it is simple to calculate the volume of the plasma fluid shift corresponding to any observed change in Hb level and PCV. As an example we have taken a typical case of a 3·4 kg. infant where the Hb after birth rises by 20% from 16·9 to 20·3 g./100 ml. and the PCV by 15% from 53 to 61%. The blood volume after birth, corresponding to a PCV of 61%, is 90 ml./kg. (Mollison *et al.*, 1950), and from this values for the plasma and red cell volumes at birth and after birth have been calculated (Fig. 5).

According to this calculation it will be seen that in a typical case such as that quoted the volume of fluid extruded from the vascular compartment will be 367-306 = 61 ml., while the plasma volume will diminish by 205-151 = 54 ml. The difference between these figures is accounted for by the 7 ml. of fluid that has shifted from the red cells, equivalent to a contraction of about 4% in the red cell volume. The 54 ml. of plasma fluid lost from the vascular compartment out of an initial plasma volume of 205 ml. represents 26% of the initial plasma volume.

The Composition of the Fluid Lost from the Vascular Compartment

Plasma being composed of electrolytes, protein and water, the question next to be answered is whether all these constituents are lost in the same proportions in which they exist in the plasma.

The percentage increase in the concentration of a solute when the volume of a solution diminishes from V_1 to V_2 is given by $100\,\frac{(1/V_2-1/V_1)}{1/V_1}$ which reduces to $100\left(\frac{V_1}{V_2}-1\right)$. If the terms V_1 and V_2 are replaced in this expression by the values for the plasma volume at birth and after birth as given in Fig. 5, this expression becomes

$$100\left(\frac{Hb_2}{Hb_1}\cdot\frac{100-0{\cdot}83\ PCV_1}{100-0{\cdot}83\ PCV_2}-1\right)$$

If now the fluid lost from the vascular compartment were protein-free, then the above expression would give the percentage increase in plasma protein concentration in terms of the changes in Hb level and PCV after birth. If, on the other hand, the fluid lost were whole plasma, there would be no change in the concentration of plasma protein. In Fig. 6 the percentage change in the plasma protein concentration, calculated on the supposition that

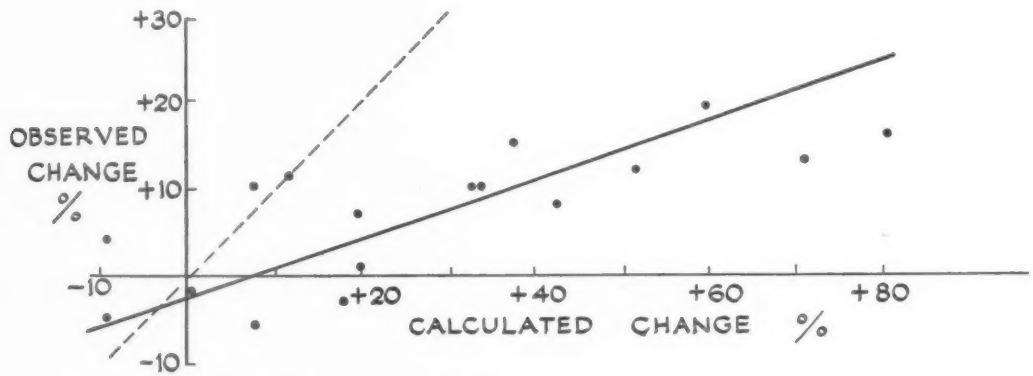


FIG. 6.—Observed change in plasma protein concentration one to eight hours after birth (17 cases), related to change calculated on the supposition that the fluid lost from the vascular compartment after birth is protein-free. Regression (continuous) line shows that observed change is about one quarter as great as calculated change.

the fluid lost is protein-free, is plotted against the observed change. It will be seen that the points mainly fall far below the broken line representing a 1:1 ratio between the calculated and the observed changes in protein concentration. The position of the regression line shows that the observed change in protein concentration is on the average only about one quarter of the calculated value, and from this fact we may infer that the protein content of the fluid which leaves the vascular compartment is roughly three-quarters that of the plasma.

In the same way, the fact that the plasma sodium shows no postnatal rise implies that any fluid lost from the vascular compartment contains about the same concentration of sodium as does plasma.

In brief, therefore, we may conclude that the fluid lost is largely whole plasma, although the small increase in plasma protein concentration indicates that some additional protein-free fluid also leaves the vascular space.

The Fluid Shift from the Red Cells. We have seen that there is generally a rise in the MCHC in the first few hours after birth, averaging 4·0%. The fact that the MCHC is appreciably higher after birth than at birth is evident also when studying the data provided by other authors who have examined both cord blood and blood taken after birth on Day 1; their results are set out in Table 2. For the four series quoted the average postnatal increase in MCHC is about 3%.

An increase of 3% in the MCHC implies a contraction in the volume of the red cell by about the same amount. We have not made direct observations to check whether in individual cases the mean corpuscular volume (MCV) decreases in parallel with the MCHC increase after birth, and in view of the inaccuracy of red cell counts it would be a laborious task to attempt. However, there is some evidence from the literature that the MCV after birth on Day 1 is, on the average, some 3% lower than at birth (Table 2).

There is thus good evidence that after birth there is a contraction in the volume of the red cells which, on the average, amounts to about 3%. This rapid decrease in volume must be largely caused by a shift of fluid from the cells.

The cause of the fluid shift from the red cells is undecided. Our data show no correlation between the increase in MCHC and the increase in Hb level after birth so that, for example, the MCHC may increase in cases where there is no postnatal Hb rise. It therefore seems that the shift of fluid from the red cells on the one hand and from the plasma compartment on the other, may prove to be independent effects.

Certainly our data do not suggest that the contraction of the red cells is a response to alteration in the osmotic pressure of the plasma, for the plasma sodium, which (with the chloride) makes the major contribution to the osmotic pressure of the plasma, shows no consistent rise after birth. The small increase noted in plasma protein concentration after birth would have only an insignificant effect on the total osmolarity of the plasma.

It is, however, known (Jacobs and Parpart, 1931) that red cells contract under the following three conditions: a rise in O_2 tension; a fall in CO_2 tension; or a shift of the *p*H towards the alkaline side; and it is possibly a combination of these three effects which causes the haematocrit of arterial blood to be about 1% lower than that of venous blood (Henderson, 1928; Hope and Verel, 1955). After birth, as soon as air-breathing begins, all three effects operate, the blood *p*H (which has the largest effect on red cell size) shifting from 7·33 to 7·43, according to Graham and Wilson (1954). It must be stressed that the gas tensions in a sample of blood examined by ordinary haematological techniques can bear little relation to those in the body. Nevertheless changes in O_2 and CO_2 tensions provide an attractive theory to explain the postnatal contraction of red cells and this possibility requires further study.

TABLE 2

CHANGE IN COMPOSITION (MCHC) AND SIZE (MCV) OF RED CELLS AFTER BIRTH

Author	Number of Cases	(1) Birth (Cord Blood)	(2) After Birth (Day 1)	Difference (1)—(2)
		Mean Corpuscular Hb Concentration (%)		
Present series	21	32·3	33·6	+4·0%
Waugh *et al.*, 1939	45	30·0	31·5†	+5·0%
Vahlquist, 1941	24	32·2	32·4	+0·6%
Guest and Brown, 1957	59	32·6	33·5	+2·7%*
		Mean Corpuscular Volume (c. μ.)		
Vahlquist, 1941	24	116·2	113·2	−3·0%
Guest and Brown, 1957	59	113	110	−2·7%*

* Differences statistically significant. † Day 2.

The Mechanism of the Postnatal Shift of Plasma. We have inferred that in a typical newborn infant birth is quickly followed by the shift of an appreciable fraction of the plasma volume, amounting in an average case to some 54 ml. of plasma. At present we can do no more than speculate about the mechanism of this substantial shift of plasma, which often begins within a few minutes of birth and appears to be complete within an hour or two.

From animal and human studies it is known that plasma fluid with varying amounts of protein is continuously flowing out of capillaries. Some of this fluid is reabsorbed on the venous side of the capillary, while the remainder, including practically all the protein, is recovered by the lymphatics and eventually returned to the circulation. It is less well known how considerable is the amount of protein which thus leaves the circulation, giving rise to a regular extravascular circulation of plasma protein via the lymphatic system, the lymph having a concentration of protein of about half that in plasma itself. In the dog it has been estimated that 50-100% of the total plasma protein leaves the circulation each day (Yoffey and Courtice, 1956).

Under physiological conditions the main variable factor determining the amount of plasma fluid leaving a capillary is the hydrostatic pressure within the vessel. The cause of the postnatal plasma shift may well therefore be sought in the drastically changed haemodynamics of the infant following birth. The re-routing of the blood stream which takes place must presumably lead to a greatly increased blood flow at some sites. At no site is such a change better defined than in the lungs. This subject is discussed by Dawes (1958) who has shown that (in the lamb) birth is followed by (i) a large fall in pulmonary arterial pressure and (ii) a large increase (perhaps threefold) in pulmonary blood flow. The very large decrease in the pulmonary vascular resistance, which these two facts together imply, points to the probability of a large rise in the pressure within the smaller pulmonary vessels. This pressure has not as yet been susceptible to measurement.

It is suggested, then, that the loss of protein-rich fluid takes place from capillaries subjected to an increased pressure following circulatory rearrangements at birth, and that the pulmonary circulation is one site where conditions are such as to favour this.

Blood Volume at Birth. Determinations of the blood volume of the newborn infant have in practice been made when the infant was a few hours old, by which time we must assume from our data that the postnatal change in plasma volume will have already taken place. This conclusion is supported by inspection of the data given by Mollison *et al.* (1950) in their paper on blood volume in newborn infants, where the (corrected) haematocrit of the normal infants studied is, with a few exceptions, well above the normal mean value of 50% for cord blood, averaging about 60%. It follows that the blood volume of the infant at birth is appreciably larger than is currently accepted. Referring, for example, to the situation represented in Fig. 5, it will be seen that the inferred blood volume *at birth* was 108 ml./kg., compared with 90 ml./kg. after birth. In this case, therefore, the blood volume at birth was 20% larger than after birth, 18% being accounted for by a larger plasma volume, and 2% by a larger red cell volume.

The Hydraemic State of the Foetus. It is clear therefore that, compared with the infant after birth, the foetus tends to be in a state of hydraemia, i.e. to have a larger plasma volume.*

The question arises as to how far the wide range over which the normal Hb level in the cord blood varies is partly a function of the degree of hydraemia. In Fig. 7 the postnatal rise in Hb level has been plotted against the cord Hb level. The regression line is shown and this is highly significant ($r = 0{\cdot}352$, $p = <0{\cdot}01$). It can be seen that there is a tendency for the largest postnatal rises in Hb level to occur amongst those infants that have a low initial level.

This is an interesting finding, because it throws some light on a fact which has puzzled us, the remarkably wide range of the normal cord Hb level, the 95% range being 13·7-20·1 g. (Marks *et al.*, 1955), with occasional values observed as low as 10·4 g. and as high as 22·0 g. The findings shown in Fig. 7 strongly suggest that the lower Hb values are the result of haemodilution. For instance, while 18 out of 92 infants were born with a cord Hb below 15 g., a few hours after birth only two infants showed so low a level.

As we have inferred that immediately after birth the plasma volume may decrease appreciably, it is of interest that there is some evidence of a similar lability of the plasma volume in the foetus before birth. Born, Dawes and Mott (1956) observed that in the foetal lamb subjected to hypoxia, as by decreasing the oxygen content of the air inspired by the ewe, there was a rise averaging 11% in the oxygen capacity (i.e. the Hb concentration) of the foetal blood, the oxygen capacity tending to return

* In contrast to this use of the term *hydraemia* in its strict sense, the term has sometimes been loosely applied to the foetus, and equally to the newborn infant, in the sense that the water and salt content of the body is high (Gans and Thompson, 1957).

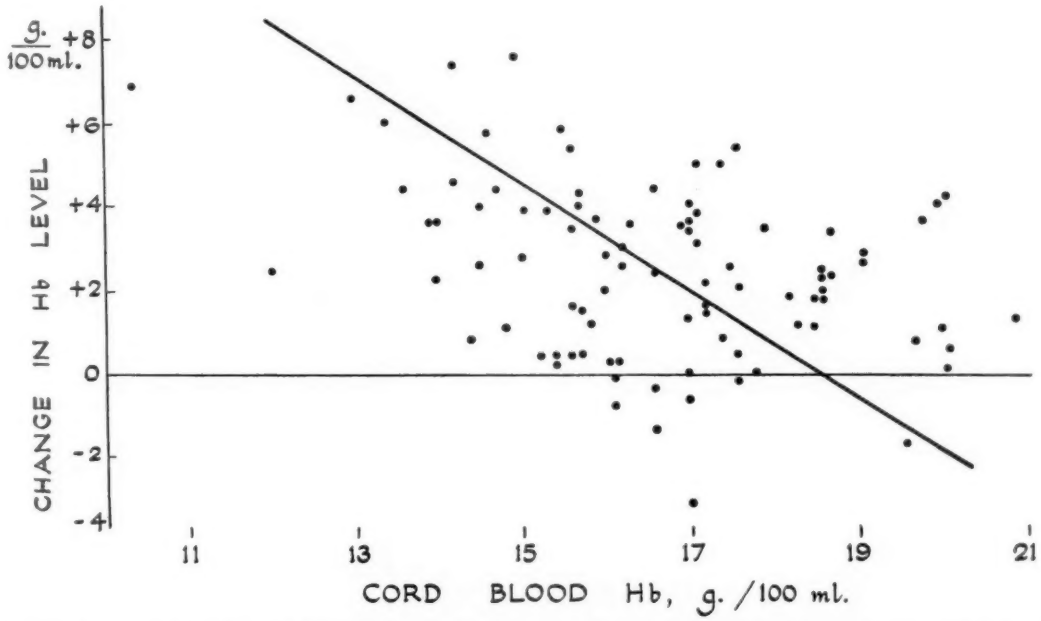


FIG. 7.—Change in Hb level one to eight hours after birth related to cord blood Hb level (92 cases). The regression line shows that the lower the cord blood Hb level, the larger the postnatal haemoconcentration effect.

to its original level after oxygenation was restored. This effect was shown not to be due to placental blood transfer and would be most readily explained on the basis of the readiness of the foetus to lose plasma from the circulation. The same explanation may account for the observation of Cook, Brodie and Allen (1957) that the mean cord blood Hb level of infants born with evidence of pre-natal hypoxia (meconium staining of nails, skin and cord) was 19·2 g./100 ml., significantly higher than the level of 17 g. in normal infants, and attributed by these authors to haemoconcentration. A similar conclusion was reached by Sjöstedt, Engelson and Rooth (1958) who found that infants showing meconium staining of the skin tended to have an abnormally low oxygen saturation of umbilical vein blood, and an abnormally high mean Hb level, 17·0 g. against the normal 16·1.

The Premature Infant and Plasma Shift. Only a few of the infants studied in this series were premature. In these few the usual tendency for the Hb level to rise strikingly after birth has been observed and it seems likely that in premature as in normal infants, birth is often followed by a shift of plasma from the circulation. A fluid shift which is evidently a physiological process in the normal infant might, however, prove an important handicap to an immature infant. The conspicuous tendency for the premature infant to develop generalized subcutaneous oedema within a few hours of birth comes to mind in this connexion, since it implies that immediately after birth there is a substantial shift of fluid from an undetermined source into the interstitial spaces. Moreover, there is some evidence to suggest that this fluid has a high content of protein, since Baar (1956) has been able at necropsy to collect oedema fluid from the leg in some premature infants. He found the protein content of this fluid to be near to that of plasma, with a concentration of from 3 to 7 g. %, and an electrophoretic pattern similar to that of plasma, save that the proportion of albumen was rather higher.

More important from the point of view of the vital functions is the part played by oedema in the pulmonary failure which is the main cause of death in the premature. From the standpoint of morbid anatomy there are two facets to these cases: resorption atelectasis, and intra-alveolar 'hyaline membrane'. The latter is only seen in infants that have survived several hours (Potter, 1952) and is probably formed from a transudate of plasma protein. We have already given reasons why the lungs are likely to be one of the sites where plasma is extruded postnatally. It is suggested that in the premature infant the normal mechanism by which the lymphatic system recovers protein leaving the circulation is overwhelmed, and that pulmonary oedema follows, with some of the protein-rich oedema fluid exuding into the alveolar space. In the average infant at

birth the lungs weigh about 60 g., compared with a volume of fluid entering the extravascular space which we have estimated in a typical case to amount to about 61 ml. Thus even a small part of this fluid, if it remained in the lungs, might be of functional significance in the immature infant.

This reasoning leads then to the view that pulmonary oedema may be the primary disturbance in the pulmonary failure which so often overtakes the premature baby within a few hours of birth, and that one factor in the pathogenesis of the oedema may be the postnatal shift of plasma from the circulation.

Summary

Immediately after birth there is a rise in concentration of Hb and in PCV in the majority of infants. The rise may be discernible within a few minutes of birth and is probably completed within an hour or two. It occurs in the absence of any transfer of placental blood.

The possible causes are considered in detail and it is concluded that the effect is brought about by a shift of fluid from the vascular compartment.

This fluid is mainly whole plasma, and its volume may amount to a large fraction of the circulating plasma at birth, a quarter or more.

In addition, there is a shift of fluid from the red cells, the volume of red cells contracting after birth by an average of 3%.

The cause of these fluid shifts is discussed and it is suggested that the plasma loss may be a consequence of the re-routing of the circulation after birth.

These results imply that the foetus *in utero* is hydraemic, i.e. has a large plasma volume.

Haemodilution is thus in part responsible for the wide range of the normal cord Hb level.

Possible clinical implications are mentioned particularly in relation to the pulmonary failure of the premature infant.

We gratefully acknowledge biochemical help given us by Professor R. A. McCance, F.R.S., Dr. R. S. Lawrie and Mr. S. H. H. Davison, and haematological facilities granted us by Dr. D. G. Chalmers.

References

Baar, H. S. (1956). Quoted by Crosse, V. M. (1957). *Ann. paediat. Fenn.*, **3**, 153.
Born, G. V. R., Dawes, G. S. and Mott, J. C. (1956). *J. physiol.*, **134**, 149.
Chaplin, H. and Mollison, P. L. (1952). *Blood*, **7**, 1227.
——, —— and Vetter, H. (1953). *J. clin. Invest.*, **32**, 1309.
Cook, C. D., Brodie, H. R. and Allen, D. W. (1957). *Pediatrics*, **20**, 272.
Dawes, G. S. (1958). In *Recent Advances in Paediatrics*, ed. D. Gairdner, 2nd ed. Churchill, London.
DeMarsh, Q. B., Windle, W. F. and Alt, H. L. (1952). *Amer. J. Dis. Child.*, **63**, 1123.
——, Alt, H. L. and Windle, W. F. (1948). *Ibid.*, **75**, 860.
Gairdner, D., Marks, J. and Roscoe, J. D. (1952). *Arch. Dis. Childh.*, **27**, 214.
Gans, B. and Thompson, J. C. (1957). *Proc. roy. Soc. Med.*, **50**, 929.
Graham, B. D. and Wilson, J. L. (1954). *A.M.A. Amer. J. Dis. Child.*, **87**, 287.
Henderson, L. J. (1928). *Blood, a Study in General Physiology*, chap. 7. Yale University Press, New Haven.
Hope, A. and Verel, D. (1955). *Clin. Sci.*, **14**, 501.
Jacobs, M. H. and Parpart, A. K. (1931). *Biol. Bull.*, **60**, 95.
Marks, J., Gairdner, D. and Roscoe, J. D. (1955). *Arch. Dis. Childh.*, **30**, 117.
Mollison, P. L., Veall, N. and Cutbush, M. (1950). *Ibid.*, **25**, 242.
Potter, E. L. (1952). *Pathology of the Fetus and the Newborn.* Year Book Publishers, Chicago.
Seip, M. (1953). *Acta med. scand.*, Suppl. 282.
—— (1955). *Acta paediat.*, **44**, 355.
Sjöstedt, S., Engelson, G. and Rooth, G. (1958). *Arch. Dis. Childh.*, **58**, 123.
Wegelius, R. (1948). *Acta paediat.*, **35**, Suppl. 4.
Yoffey, J. M. and Courtice, F. C. (1956). *Lymphatics Lymph and Lymphoid Tissue*, 2nd ed., chap. 2. Arnold, London.

THE PLASMA AMINO ACIDS IN MALNUTRITION:

PRELIMINARY OBSERVATIONS*

BY

ROLAND G. WESTALL,† ELLEN ROITMAN, CARMEN DE LA PEÑA, HOWARD RASMUSSEN, JOAQUIN CRAVIOTO, FEDERICO GÓMEZ and L. EMMETT HOLT, JR.

From the Department of Pediatrics, New York University College of Medicine and the Group for Research in Infant Malnutrition, Hospital Infantil, Mexico

(RECEIVED FOR PUBLICATION MAY 20, 1958)

It is generally appreciated that malnutrition is the world's foremost paediatric problem. Directly or indirectly it makes the major contribution to child mortality. Nevertheless, it remains an entity which is not well defined, or, more accurately, a combination of entities only some of which are well defined. We recognize the effects of certain specific food factors, the accessory food factors or vitamins, and we have some knowledge of changes brought about by deficits of the inorganic components of diet. The effects of caloric deficits, of protein deficits and of possible deficits of specific amino acids have, however, been difficult to separate. Protein and caloric deficits are commonly associated and the existence of syndromes of specific amino acid deficiency has not been definitely established although there is some evidence that they exist.

It appears that the more exact definition of this area of deficiency will depend on chemical studies. We already know that plasma protein levels fall in protein deficiency, particularly the albumin fraction, and that blood urea is also reduced. We know that certain protein enzymes in the blood and the liver (Waterlow and Patrick, 1954; Burch, Arroyave, Schwartz, Padilla, Béhar, Viteri and Scrimshaw, 1957) are diminished. It has seemed to us that observations on the free amino acids of the plasma might be of help in delineating specific entities within this area. The free amino acid level of the plasma might prove to be a more significant index of protein adequacy than other criteria that have been applied and the plasma free amino acid pattern, even if it could not be correlated with symptoms of specific amino acid deficiency, might yet be of considerable value in pointing to limiting amino acids and thus providing a sounder basis for dietary supplementation than has hitherto been available. Preliminary studies of urinary amino acids in kwashiorkor by members of our group (Cheung, Fowler, Norton, Snyderman and Holt, 1955), by Maggioni (1957) and by Hansen (1957) had shown abnormal amino acid excretion patterns, but since these might have been of renal origin it seemed preferable to explore the blood patterns rather than to extend the observation of urinary amino acids.

The observations reported here were made on four children admitted to the nutrition ward of the Hospital Infantil, Mexico City, with severe clinical evidences of malnutrition. There was no clinical evidence of vitamin deficiencies. They were all thought to be suffering from caloric deficiency as well as from protein deficiency, but the severity of the protein deficiency was much more marked in one of these subjects (M.M.C.) than in the other three. Only this patient exhibited the oedema and skin pigmentary changes typical of kwashiorkor. The serum of these four patients was studied initially and at intervals during the phase of recovery by the technique of column chromatography. Individual case histories are given below.

Case Histories

Case 1. R.A.M. (231195), a boy aged 1 year 11 months, was admitted to the Hospital Infantil on June 11, 1957, suffering from diarrhoea. No reliable information was obtainable about his past history or present illness. He was grossly undernourished, though not dehydrated, and the panniculus adiposus was minimal. His weight was 6·7 kg., height 71 cm. The skin showed somewhat deeper pigmentation on the dorsal surfaces of the hands and feet than elsewhere, but there were no discrete lesions. The liver was palpable 2 cm. below the costal margin in the mammary line. No evidence of infection was discovered, and the tuberculin reaction was negative. The bone age corresponded to that of a child of 6 months, according to Todd's Atlas (1937).

* This study was supported in large part by a grant from E. I. du Pont de Nemours and Co., Wilmington, Del.

† Present address: Medical Unit, University College Hospital Medical School, London.

The diarrhoea proved not to be severe, only five to six stools a day being passed. No pathogens were isolated on culture, but a microscopic examination revealed ova of *Hymenolepsis nana* and cysts of *Giardia lamblia*. The appetite was good and the patient took increasing amounts of the regular ward diet which consisted of whole cow's milk with supplements of bread, rice, banana, liver, lean beef and beans.

The samples of blood serum for amino acid assay were taken on the dates shown in Table 1.

During the period in hospital there was a gradual improvement in the child's general appearance. During the first four days he lost some weight, but after this there was a steady gain which reached 8·7 kg. on August 7, the day of discharge. Evidence of the 'recovery syndrome' (Gómez, Ramos Galván and Cravioto Muñoz, 1952) was first noted on June 20. The abdomen was enlarged with signs of free fluid in the abdominal cavity and prominent superficial abdominal veins. The liver edge at this time was felt 4 cm. below the costal margin. A blood examination at this time showed:

R.B.C. 3·3 millions, haematocrit 28%, haemoglobin 6·8 g., W.B.C. 18,000 (P. 57%, E.4%, L. 35%, M. 4%). The urine was normal. The stools at this time showed only *Giardia* cysts. Total plasma proteins 7·43 g. %; albumin 3·82 g., total globulin 3·61 g. ($\alpha = 0{\cdot}7$, $\beta = 1{\cdot}59$, $\gamma = 1{\cdot}32$).

By July 7, hepatomegaly and ascites had further increased as had the prominence of the abdominal vein, and examination of the blood showed the following:

R.B.C. 3·1 millions, haematocrit 26%, haemoglobin 7·3 g., W.B.C. 8,000 (P. 46%, E. 3%, B. 1%, L. 48%, M. 2%). Total protein 7·60 g., albumin 3·63 g., total globulin 3·97 g. ($\alpha = 1{\cdot}55$, $\beta = 1{\cdot}15$, $\gamma = 1{\cdot}27$).

Between July 7 and August 7 evidence of the recovery syndrome diminished markedly, but did not altogether disappear, and a blood study on July 25 showed:

R.B.C. 3·4 millions, haematocrit 29%, haemoglobin 7·3 g., W.B.C. 6,500 (P. 23%, E. 5%, L. 69%, M. 3%).

Case 2. E.F.A. (231172), a boy of 2½ years, was admitted to the Hospital Infantil on June 10, 1957, with malnutrition which had developed since weaning at 2 months of age. For a time he had been fed on a mixture of dilute cow's milk and tea, but on this he had not thrived and had suffered attacks of diarrhoea associated with fever and loss of weight. These attacks had recurred at intervals up to the time of admission. After the age of 1 year he had received almost no milk, his diet consisting of corn meal, beans, bread and noodle soup.

On admission the patient was found to be markedly undernourished. His weight was 5·7 kg., height 71 cm. The anterior fontanelle was still open (2 × 2 cm.). The skin showed no oedema, but there was a striking loss of subcutaneous fat. There was some excessive diffuse pigmentation, particularly on the extremities, but no discrete lesions. The liver edge was not palpable. Bone age corresponded to that of a child of 6 months (Todd, 1937).

The patient was given the regular ward diet. For the first two days some weight was lost but thereafter there was a steady gain, reaching 6·3 kg. on June 21, 6·8 kg. on July 10 and 7·1 kg. on August 7, the day of discharge. He had improved steadily up to June 25 when he developed bronchopneumonia which necessitated the use of antibiotics and oxygen. This attack was, however, brief and did not interrupt his weight gain. By June 28 the pulmonary signs had virtually disappeared. Evidence of the recovery syndrome was noted on July 8: the abdominal veins were engorged and the liver was now palpable 1½ cm. below the xyphoid. By August 1 these signs were receding.

Routine studies of the blood morphology and of the plasma proteins were made on three occasions:

On June 21: R.B.C. 3·6 millions, haematocrit 31%, haemoglobin 8·9 g., W.B.C. 12,000 (P. 63%, E. 2%, L. 30%, M. 5%). Total plasma proteins 6·9 g. %, albumin 3·31 g., total globulins 3·59 g. ($\alpha = 0{\cdot}44$, $\beta = 1{\cdot}99$, $\gamma = 1{\cdot}11$). Urine was normal. Stools showed cysts of *Giardia lamblia*.

On July 8: R.B.C. 3·1 millions, haematocrit 26%, haemoglobin 8·2 g., W.B.C. 6,000 (P. 36%, B. 8%, L. 54%, M.2%). Total protein 6·35 g. %, albumin 3·63 g., globulin 2·72 g. ($\alpha = 0{\cdot}73$, $\beta = 0{\cdot}72$, $\gamma = 1{\cdot}57$).

On August 2: R.B.C. 4·7 millions, haematocrit 40%, haemoglobin 12 g., W.B.C. 9,700 (P. 56%, E. 4%, L. 38%, M. 2%). Total plasma protein 6·07 g. %, albumin 3·09, total globulin 2·98 ($\alpha = 1{\cdot}54$, $\beta = 0{\cdot}36$, $\gamma = 1{\cdot}08$).

Case 3. F.C.G. (231194), a boy of 3 years, was admitted to the Hospital Infantil on June 11, 1957, with a history of bouts of diarrhoea since the age of 8 months. The attack for which he was admitted had lasted 15 days, and was precipitated by measles. The diarrhoea was not severe, only five to six stools a day being passed.

On admission the patient was markedly wasted, his weight being 8·0 kg., height 80 cm. The skin showed general pigmentation but no discrete lesions. There was no oedema. The liver was not palpable. Laboratory examinations on admission showed a normal urine. No pathogenic bacteria were cultivated from the stools, but cysts of *Giardia lamblia* were present. The blood showed: R.B.C. 3·6 millions, haematocrit 31%, haemoglobin 10·3 g., W.B.C. 2,000 (P. 38%, L. 60%, M. 2%). Total protein 5·35 g. %, albumin 3·12, globulin 2·23 ($\alpha = 0{\cdot}64$, $\beta = 0{\cdot}32$, $\gamma = 1{\cdot}27$).

For the first five days in hospital the patient was given a diet of corn meal and fish flour; for a second five-day period he was fed with corn and beans supplemented with glycine; after this he received the regular ward diet, similar to that given to the two patients previously described. Loss of weight was confined to the first 24 hours after admission. After this he gained steadily, reaching a weight of 9·6 kg. on August 6, the day of discharge. The diarrhoea proved to be mild, subsiding after the first few days, and the general appearance improved steadily.

The signs of the recovery syndrome, enlargement of the abdomen with hepatomegaly and engorgement of the superficial abdominal veins, were first noted on

July 8. The liver edge was felt 5 cm. below the costal margin and there was some evidence of free fluid in the abdominal cavity. On August 1 these signs, though still detectable, were definitely on the wane.

A routine blood examination on July 5 showed the following: R.B.C. 3·8 millions, haematocrit 32%, haemoglobin 9·1 g., W.B.C. 6,700 (P. 44%, E. 1%, L. 53%, M. 2%). Total proteins 7·02 g. %, albumin 3·94, total globulin 3·08 ($\alpha = 0·91$, $\beta = 0·52$, $\gamma = 1·65$).

Case 4. M.M.C. (231196), a girl of 1 year 7 months, was admitted to the Hospital Infantil on June 11, 1957, with a history of diarrhoea and vomiting for two months. For the preceding two weeks she had been weak and apathetic, and there was loss of appetite and oedema of the legs. Her diet had consisted largely of beans and noodle soup, but some milk had been given two to three times a week, possibly as much as 10 oz. on the days it was given. She was markedly undernourished, weight being 7·4 kg., height 77 cm. Discrete skin lesions, hyperpigmented and scaly, and typical of kwashiorkor, were present on the arms and legs and to some extent on the trunk. The hair was dry and relatively depigmented; it was easy to pull out. The tongue was red with hypertrophy of the fungiform and atrophy of the filiform papillae. There was tremor of the arms and a definite carpopedal spasm.

The patient was given calcium intravenously with prompt disappearance of the tetanic symptoms. The routine ward feeding was given from the start. Diarrhoea and vomiting were not observed in the hospital, and no parasites were found in the stools. The appetite and general well-being improved steadily. Weight was lost during the first two weeks in hospital as the oedema disappeared, the lowest point, 6·6 kg., being reached on June 25. After this the weight steadily increased to 8·6 kg. on August 9, the day of discharge. On June 27 it had increased and engorgement of the abdominal veins was observed. There was evidence of free fluid in the abdominal cavity on July 8. Defervescence of all these manifestations was clearly evident during the first days of August.

Routine blood studies were made on four occasions with the following results :

Date	R.B.C. (millions)	Hct. (%)	Hb (g.)	W.B.C.	Polys. (%)	Total Protein (g.%)	A/G	Globulins α	Globulins β	Globulins γ
June 12	3·2	—	9·1	6,200		3·76	1·56/2·20	0·69	0·68	0·83
„ 24	3·3		9·5	15,600	83	5·03	2·34/2·69	0·71	0·14	1·24
July 4						6·75	3·75/3·00	1·03	1·17	0·80
„ 31	4·5	38	11·2	15,600	28					

Experimental Procedure

Blood samples were taken at four different times from each patient, the first soon after admission, another sample about eight to nine days later, a further sample at the 25-30 day period and a final sample at eight to nine weeks. Approximately 20 ml. of blood, taken at least three hours after the last meal, was drawn with a heparinized syringe from the jugular vein. The sample was centrifuged and 10 ml. was shaken with 50 ml. of 1% picric acid following precisely the details described by Moore and Stein (1954) for deproteinizing the plasma and removing the excess picric acid. The conditions used for the column chromatographic analyses again followed closely those described by Stein and Moore (1954) except that Dowex 50 × 5% cross-linked resin was used instead of a blend of the 4 and 5% cross-linked resin recommended by the above authors.

Results

The plasma amino acid concentrations in mg./100 ml. of plasma of the four patients, initially and during recovery, are given in Table 1. A range of normal values is also included. These normal values are derived partly from an analysis of plasma amino acids of normal children of similar age, which we have performed in the Pediatric Department of Bellevue Hospital, New York, and partly from the few figures available from published reports (Huisman, 1954; Stein and Moore, 1954; Iber, Rosen, Levenson and Chalmers, 1957). Throughout we were unable to detect any tryptophan in the plasma of the four children in this study nor was this amino acid found in the plasma of our normal children of this age group. We found it in older children and Stein and Moore reported a well-defined peak in their diagram of adult plasma. However, the latter authors reported that some loss of tryptophan can occur during the analytical procedure. We can only assume, therefore, that in children of 1 to 3 years the plasma concentration of tryptophan is so low that its presence is masked by the small losses inherent in the method.

The figures for ammonia and urea are also given since these are also determined by this method. The ammonia figure does not represent true ammonia present in plasma at the time of sampling since it is well known that ammonia is liberated and the concentration builds up during the deproteinization procedure. The figure for total amino acids represents the sum of the amino acids which were determined individually.

The changes observed in the plasma of the three patients who showed neither oedema nor appreciable skin changes were fairly consistent. They were least conspicuous in R.A.M. who showed the least reduction in weight and who during treatment failed to increase his total amino acid concentration

TABLE 1
AMINO ACID CONCENTRATION (MG./100 ML. BLOOD PLASMA)

Amino Acid	F.A.E. (2½ yr.) ♂ Day 1	11	30	60	F.G.C. (3 yr.) ♂ Day 1	9	25	55	R.A.M. (1 yr. 11 m.) ♂ Day 1	8	25	55	M.M.C. (1 yr. 3 m.) ♀ Day 1	9	26	55	Normal Range
Alanine	0·3	3·7	1·9	1·3	1·6	10·0	6·2	3·6	3·3	2·3	2·1	2·4	0·7	5·4	2·5	3·3	3·0–4·8
Aspartic acid	0·03	0·0	0·07	0·20	0·02	0·0	0·20	1·0	0·1	0·0	0·4	0·1	0·04	0·0	0·2	0·5	0·1–0·2
Asparagine + glutamine	2·1	4·0	2·8	1·5	2·6	5·0	3·2	3·0	3·9	3·9	1·5	4·0	2·0	7·7	1·7	2·3	3·0–5·0
α-aminobutyric acid	0·05	0·1	0·1	0·2	0·08	0·0	0·0	0·4	0·0	0·0	0·0	0·0	0·0	0·1	0·1	0·4	0·2–0·4
Arginine	0·4	0·9	0·4	—	0·4	0·8	0·5	0·4	0·4	0·6	0·6	0·4	—	0·5	0·7	0·9	0·8–1·4
Cystine	0·6	0·7	0·8	0·6	0·6	0·6	1·5	0·4	1·0	0·7	4·0	0·4	0·1	0·2	1·6	0·4	1·0–1·5
Glutamic acid	1·3	1·3	0·8	1·8	0·8	0·7	0·1	1·8	0·9	0·7	0·1	1·9	0·6	1·7	1·7	2·0	1·0–1·5
Glycine	2·2	3·0	1·3	1·2	1·9	0·6	0·7	2·1	1·9	1·7	0·6	1·8	0·8	1·3	1·8	1·5	1·0–2·0
Histidine	0·8	0·8	1·0	—	1·0	0·9	1·0	1·5	0·7	0·0	1·2	1·2	1·2	1·7	1·4	1·2	1·0–1·7
Isoleucine	0·5	1·0	0·7	1·0	0·7	0·8	1·2	0·9	0·7	0·7	0·7	0·9	0·8	0·7	0·7	1·5	0·8–1·5
Leucine	0·7	1·7	1·1	1·1	0·4	1·3	2·2	1·5	1·0	1·3	1·1	1·7	0·4	1·2	1·3	2·0	1·5–2·9
Lysine	0·9	2·3	1·0	—	0·9	2·7	1·4	2·1	0·7	1·7	2·3	1·9	0·6	3·6	2·6	2·4	1·5–2·4
Methionine	0·1	0·3	0·1	0·1	0·15	0·5	0·4	0·4	0·4	0·2	0·2	0·4	0·2	0·2	0·3	0·1	0·3–0·6
Ornithine	0·3	0·8	0·3	—	0·3	0·6	0·4	0·5	0·3	0·4	0·0	0·6	0·1	0·8	1·1	1·1	0·6–0·8
Proline	0·8	2·4	1·3	1·1	1·6	2·2	1·7	2·4	2·2	1·4	2·1	2·4	0·7	3·1	1·8	3·1	1·5–3·0
Phenylalanine	0·7	1·3	0·7	—	0·7	1·2	2·3	1·4	1·2	1·5	1·3	0·9	0·3	0·5	0·7	1·9	1·0–1·7
Serine	0·7	1·5	1·1	1·1	1·2	4·6	1·9	1·2	1·4	1·4	1·3	1·4	0·6	2·2	1·2	1·6	1·3–2·2
Taurine	1·3	0·2	0·4	0·6	0·6	1·5	0·8	0·3	0·3	0·9	0·5	0·3	0·2	0·5	2·1	0·9	0·9–1·8
Threonine	0·3	1·0	0·6	1·0	0·4	1·4	1·4	0·9	0·5	0·5	1·1	0·9	0·2	1·5	1·2	1·6	1·2–1·6
Tyrosine	0·7	1·3	0·9	—	0·6	0·6	1·4	1·7	1·3	1·1	1·0	1·5	0·1	0·2	0·7	1·9	1·5–2·3
Valine	1·2	3·0	1·7	2·1	0·8	1·6	3·3	1·9	1·7	1·7	2·9	1·7	0·2	1·7	2·0	2·7	2·0–3·0
Total	15·98	31·30	19·07	14·9*	17·35	37·60	31·80	29·40	23·9	22·5	25·0	26·8	9·84	34·7	27·4	33·3	28–33
Ammonia	0·23	0·22	0·05	—	0·26	0·20	0·30	0·4	0·18	0·3	0·3	0·5	0·28	0·38	0·07	0·20	
Urea	17·5	13·3	11·2	2·5	9·33	13·1	11·6	1·5	7·3	14·1	2·7	1·4	0·7	4·5	28·6	15·0	20–30

in the plasma. The general findings were as follows:

R.A.M., the least affected of the group, showed only a slight reduction in total amino acids although the urea concentration was low. Some of the amino acids were just within normal range, but significant reductions were present in the concentrations of threonine, taurine, lysine, leucine, ornithine and arginine. The eight-day specimen showed approximately the same picture except that aspartic acid was absent. In the two later specimens a fall in urea was noted, a phenomenon seen in the other subjects in association with the recovery syndrome.

F.A.E. and F.C.G. showed the same changes as R.A.M. and, in addition, several others. Their total amino acid concentrations were substantially reduced and several other individual amino acids were distinctly low in the initial specimen: alanine, aspartic acid, isoleucine, methionine, phenylalanine, tyrosine and valine. In the second specimen, the total amino acids had returned to normal as had the individual amino acids with the exception of aspartic acid and tyrosine. The tyrosine was slow in returning to normal and aspartic acid was absent in the eight-day specimen but returned later. Again the later specimens showed a reduction in urea.

M.M.C., the one who exhibited oedema and the typical kwashiorkor skin changes, showed the most profound changes in the plasma amino acids. The blood urea was extremely low and the total free amino acids were roughly one third of the normal value. Virtually all the amino acids were reduced to some extent with the exception of glycine, histidine and isoleucine. The most striking reductions were observed in the case of tyrosine, valine and cystine (to one tenth or less of the normal level). Substantial reductions (to one third or less of the normal level) were noted also in the concentrations of ornithine, threonine, taurine, leucine, alanine and phenylalanine. The levels of the remaining amino acids were reduced but less spectacularly so. The specimen taken nine days later showed a marked rise in the total amino acid figure to a level somewhat above normal. The increase was largely brought about by an abnormally high concentration of alanine and serine but at the same time the levels of the majority of the amino acids, which had been low, had returned to normal. Exceptions were phenylalanine and cystine and, most markedly, tyrosine. The subsequent specimens remained normal in respect of the amino acid concentration but tyrosine was particularly slow in reaching a normal level. Again, blood urea was low in the final specimen.

Discussion

A definite interpretation of these preliminary findings must await confirmative studies. At the most, the present data can be regarded only as suggestive. As regards the most limiting amino acid

in the indigent Mexican diet, as far as it can be judged from these results which do not permit an evaluation of tryptophan in this respect, arginine, ornithine and threonine would seem to be consistently affected even in mild cases, and leucine, valine, lysine and tyrosine thereafter. Urea is also consistently low. Methionine, phenylalanine and alanine, although not so affected in the milder cases, were markedly affected in the more severe case with typical kwashiorkor symptoms. Here also, the cystine and tyrosine concentrations were particularly low. From the above data it would be difficult to incriminate any one particular amino acid as being limiting in the Mexican diet.

With regard to imbalance of amino acids in the plasma of these patients, it follows that some imbalance must exist since the levels of some amino acids are more affected than others. However, there was certainly no abnormally high concentration of any one amino acid and the only general comment is that the glycine concentration was consistently higher than the alanine level in the initial samples whereas the reverse ratio applies in all normal plasmas.

We are, however, impressed with the possibility that the low protein diet, particularly in the case of the severely affected patient, M.M.C., interfered with the development and activity of enzymes concerned with the metabolism of amino acids. The low concentrations of arginine and ornithine suggest some interference with the urea cycle. The low concentration of cystine seen in M.M.C. suggests a deficiency in its synthesis from methionine. Even more striking in this patient is the reduced level of tyrosine as compared with phenylalanine which supports the suggestion previously made on excretion data (Cheung *et al.*, 1955) that there is this condition, a deficiency of the enzyme which converts phenylalanine into tyrosine, phenylalanine hydroxylase.* A deficiency of this enzyme had been postulated by an increased excretion of phenylalanine in the urine, a phenomenon also noted by Maggioni (1957). The slow recovery of the blood tyrosine level of our patient is also worthy of comment. It may be that assays of this enzyme will prove useful in characterizing protein deficiencies.

The marked drop in the plasma urea concentration two months after treatment began puzzled us greatly, coinciding as it appeared to do with the presence of the recovery syndrome (Gómez *et al.*, 1952). Conceivably this might be due to impaired deamination. It might also be due to some re-utilization of urea although why this should occur so late is not easy to understand. A more likely explanation would be that the recovery growth spurt causes an extremely high utilization of protein and consequently little nitrogen is available for excretion. This argument can be supported to some extent by the low level of plasma taurine in the three patients with low blood urea at this time. Dent (personal communication) points out that in a number of disease states where the utilization of protein is poor and the subject is also in negative nitrogen balance owing to excessive enzymic breakdown of body protein, the excretion of taurine, which is derived from cystine or cysteine, is high. He believes also that the converse is true and that with high utilization of protein there is little wastage of cystine and consequently a low excretion of taurine. Unfortunately, in this series of cases we were unable to cope with equivalent analyses of urine but it is now obvious that in subsequent studies it would be desirable to carry out amino acid analyses on the urine as well as the plasma.

In considering the levels of the various amino acids in the initial samples of plasma we can say without doubt that they are, in general, much below normal. To go beyond that we find ourselves at a loss as to what further inferences can be drawn as we lack the basic knowledge. For instance, in the absence of an adequate protein intake at what level of plasma amino acid concentration does the body call upon endogenous proteins to reinforce the plasma amino acids? Further, once the endogenous protein available is used up how far can the plasma amino acid level fall before the vital organs are affected? It is difficult to see how this knowledge can be adequately answered by work on experimental animals, particularly as in kwashiorkor we are dealing with children in a narrow age group. An extensive study of kwashiorkor patients, including those with the mildest and the severest forms, would, in time, give us the answer to these questions.

Summary

A study is reported of the free amino acids of blood plasma in four patients suffering from severe malnutrition. Three of these showed no specific clinical symptoms of protein deficiency, whereas the fourth exhibited oedema and skin lesions typical of kwashiorkor, and can hence be regarded as a well defined type of protein deficiency.

A tendency to aminoacidopenia was found in all four patients, involving certain essential as well as certain unessential amino acids. It is suggested that

* Since this paper was written we have had the opportunity of studying the blood of three other patients with the frank kwashiorkor syndrome, one from French West Africa and two from Nigeria. The findings, which will be reported elsewhere, showed certain differences from those reported here, but the exceptionally low tyrosine value was consistently found.

these changes are best interpreted on the basis of defective enzyme systems.

Particular attention is called to evidence pointing to a deficiency of the enzyme phenylalanine hydroxylase in the frank kwashiorkor syndrome.

REFERENCES

Burch, H. B., Arroyave, G., Schwartz, R., Padilla, A. M., Béhar, M., Viteri, F. and Scrimshaw, N. S. (1957). *J. clin. Invest.*, **36**, 1579.

Cheung, M. W., Fowler, D. L., Norton, P. M., Snyderman, S. E. and Holt, L. E. (1955). *J. trop. Pediat.*, **1**, 141.

Dent, C. E. Personal communication.

Gómez, F., Ramos Galván, R. and Cravioto Muñoz, J. (1955). *Pediatrics*, **10**, 513.

Hansen, J. D. L. (1957). Proceedings of Conference held at Princeton, 1955. Josiah Macy Jr. Foundation, p. 42.

Huisman, T. H. J. (1954). *Pediatrics*, **14**, 245.

Iber, F. L., Rosen, H., Levenson, S. M. and Chalmers, T. C. (1955). *J. Lab. clin. Med.*, **50**, 417.

Maggioni, G. (1957). *Moderne Probleme der Pädiatrie*, II, 161. Karger, Basel.

Moore, S. and Stein, W. H. (1954). *J. biol. Chem.*, **211**, 893.

Stein, W. H. and Moore, S. (1954). *Ibid.*, **211**, 915.

Todd, T. W. (1937). *An Atlas of Skeletal Maturation*. 1. The Hand. C. V. Mosby, St. Louis, Mo.

Waterlow, J. C. and Patrick, S. J. (1954). *Ann. N.Y. Acad. Sci.*, **57**, 750.

HYPOCALCAEMIC TETANY DEVELOPING WITHIN 36 HOURS OF BIRTH*

BY

W. S. CRAIG and M. F. G. BUCHANAN

From the Department of Paediatrics and Child Health, University of Leeds

(RECEIVED FOR PUBLICATION MAY 16, 1958)

Introduction

Paediatric literature contains many brief references to the occurrence of neonatal tetany. Clinical signs developed in the first week of life in only a small proportion of the recorded cases. The number of infants reported as showing signs of hypocalcaemic tetany within a few hours of birth is extremely small. Willi (1939) described eight and Bakwin (1937) four babies in whom tetany developed on the first day of life. In a more recent comprehensive study of the causes of tetany in infancy and childhood Gribetz (1957) uses the term 'first day tetany' when referring to the biochemical imvestigations of Gittleman and his co-workers. Gittleman, Pincus, Schmerzler and Saito (1956) made a special study of calcium levels on the first day of life and found low serum calcium levels with significant frequency in premature infants and in babies born at term to mothers with a history of abnormal pregnancy and/or labour.

In October, 1956, the occurrence of violent neuromuscular hyperexcitability in the day-old baby of a diabetic mother in the Leeds Maternity Hospital aroused suspicions of tetany. The baby's serum calcium was 6·2 mg. per 100 ml. Within a period of one month similar findings were encountered in the newly born babies of two other mothers with diabetes mellitus. A detailed clinical study of babies newly born to diabetic mothers was commenced. As the study proceeded a resemblance was noted between the movement pattern in these babies and that seen in a number of newly born premature infants of healthy mothers. The resemblance was one of quality rather than severity. Low serum calcium levels were obtained in a high proportion of the premature babies with neuromuscular hyperactivity. A decision was therefore made to extend the investigation to include all babies manifesting neuromuscular hyperexcitability shortly after birth.

The primary purpose of this article is to present the findings in connexion with 26 babies in whom hypocalcaemic tetany developed within 40 minutes to 36 hours of birth. As a corollary to the main study findings are also given in infants who in the first 36 hours of life either (*a*) showed clinical signs suggestive of tetany in the absence of a low serum calcium level (seven infants), or (*b*) presented no abnormal clinical features despite the presence of a low serum calcium level (two infants).

Clinical Material

Of the 35 babies included in the total study, 30 were born in the Leeds Maternity Hospital, three in St. Mary's Hospital, Leeds, and two at home prior to admission to Leeds Maternity Hospital. For the purposes of the study tetany was considered to be present if exaggerated neuromuscular hyperactivity was associated with a total serum calcium level of 8 mg. per 100 ml. or less. Total serum calcium determinations were obtained by the permanganate titration method. Ultrafilterable calcium levels were estimated in a limited number of cases. The method employed was the one described by Lavietes (1937) and used by Hopkins, Howard and Eisenberg (1952). The decision to regard a total serum calcium of 8 mg. (and less) per 100 ml. as low takes account of the recorded opinions of other workers. Bakwin (1937), Willi (1939) and Kendig (1942) consider that a serum calcium level below 8 mg. per 100 ml. provides confirmatory evidence of a clinical diagnosis of tetany. In their comprehensive laboratory study Gittleman *et al.* (1956) regarded total serum calcium levels under 8 mg. per 100 ml. as low. Studying a series of 55 healthy newborn babies Mitchell and Stevenson (1932) found a serum calcium level of under 8 mg. per 100 ml. in only one infant. In another control series of 48 healthy newborn babies investigated by Dodd and Rapoport (1949) the serum calcium level was above 8 mg. per 100 ml. in every instance.

* Based upon a paper given at the Annual Meeting of the British Paediatric Association, 1958, and illustrated by a cinematograph film.

Clinical Observations

I. Hypocalcaemic Tetany (26)

(1) The Clinical Picture. Neuromuscular hyperactivity associated with hypocalcaemia was noted in 26 infants. All infants were under observation while being nursed in incubators. Details concerning these babies are given in Table 1. In each case signs were first noted within 36 hours of birth. The severity of the clinical picture varied, being violent in seven, pronounced in 14 and mild in five babies.

TABLE 1

HYPOCALCAEMIC TETANY WITHIN 36 HOURS OF BIRTH

Neuromuscular Excitability			Total Serum Calcium* (mg./100 ml.)	
Degree	Age at Onset (hr.)	Duration (hr.)	On 2nd day of life	Lowest estimate in 1st 72 hr. of life
Violent (7)	22	108	2·6	2·6
	10	120	5·1†	5·1
	1	130	6·0	6·0
	24	32	6·2	6·2
	10	62	7·1	6·8
	8	30	7·3	6·8
	1	21	8·0	8·0
Pronounced (14)	19	36	6·1	5·7
	4	77	6·4	6·4
	7	72	6·7	4·6
	36	24	6·8	6·8
	17	52	6·8	6·8
	24	36	7·2	6·8
	6	18	7·3	7·3
	22	48	7·3	6·5
	22	60	7·4	7·4
	14	72	7·6	7·6
	18	32	7·8	7·8
	13	90	7·9†	7·9
	6½	130	8·0	8·0
	24	48	8·0	8·0
Mild (5)	33	24	5·6	5·6
	17	24	7·0	7·0
	24	36	7·1	7·1
	4	40	7·8	7·3
	22	24	7·9	7·3

* The concentration of calcium in the cord blood was estimated in 21 cases. The lowest level obtained was 9·9 mg., the average being 10·5 mg. per 100 ml.

† Given exchange transfusion on account of haemolytic disease.

A detailed description of the signs in certain of the infants included in this series has already been recorded (Craig, 1958). Clinical signs were characterized by an abnormal movement pattern consisting of alternating phases of generalized hyperactivity and silent, exhausted immobility. Hyperactivity took the form of coarse waving movements of the limbs, agitated vibrations of the hands and fine persistent tremors of the fingers. The toes were splayed out. In severe cases the cry was squeaky and hollow and the expression one of distress. In all babies there were periods of apnoea and during phases of severe agitation the entire body acquired a grey cyanotic tinge. Cyanotic apnoea not due to aspiration of fluid was particularly liable to occur at the time of feeds.

Other features noticed in a proportion of cases were tachypnoea, tachycardia, repetitive blinking of partially closed eyelids, and the ejection of minute meconium stools. Trousseau's and Chvostek's signs were not present but in four babies a position simulating pedal spasm was assumed following local pressure on the lower limbs. Laryngeal stridor was a feature in two babies. Phases of neuromuscular hyperexcitability occurred spontaneously, and as a result of stimulus by touch, noise, vibration and light. Repeated stimuli resulted in temporary diminution of response. Spontaneous phases recurred at short intervals over varying periods of 12 hours to six days. There were convulsions in four infants. Details of the abnormal movement pattern in these 26 babies are summarized in Table 2.

TABLE 2

CLINICAL FEATURES OF HYPOCALCAEMIC TETANY WITHIN 36 HOURS OF BIRTH

26 INFANTS

Generalized neuromuscular hyperexcitability	26
Convulsions	4
Simulation of carpo-pedal spasm	4
Spontaneous attacks of apnoea and/or cyanosis	13
Apnoea stimulated by feeding	13
Frequent regurgitation	10
Hollow or squeaky cry	13
Stridor	2
Tachycardia	4
Tachypnoea	5
Complete disappearance of abnormal clinical features	26
Total serum calcium level of 10 mg. or more per 100 ml. on or before seventh day of life	26

None of the babies was given cow's milk or alkaline therapy prior to the onset of tetany and at no time did any suffer from vomiting, diarrhoea or excessive fluid loss.

(2) The Biochemical Picture. Biochemical findings in the 26 examples of hypocalcaemic tetany are given in Table 3.

(3) The Influence of Calcium Therapy. Calcium was given to five babies with clinically violent tetany. The results were not uniformly conclusive. Rapid improvement followed oral administration in one infant, and intramuscular administration in another. In the remaining three babies the impression was gained that combined intramuscular and oral therapy may have accelerated the disappearance of clinical signs of tetany.

(4) The Obstetric Background. Details are summarized in Table 4. There were two cases of placenta praevia, one being associated with toxaemia. Delivery was spontaneous in 15 cases and by caesarean section in 11 cases.

TABLE 3

BIOCHEMICAL FINDINGS DURING FIRST 4 DAYS OF LIFE IN HYPOCALCAEMIC TETANY: 26 INFANTS

Baby	Day	Total Ser. Ca. (mg./100 ml.)	Ultrafilt. Ca. (mg./100 ml.)	Phosphorus (mg./100 ml.)	Alk. Phosph. (Units*/100 ml.)	Blood Urea (mg./100 ml.)
			(A) Violent Hyperexcitability (7)			
Ma	2 4	6·2 10·0	— —	— 3·0	— 23	— 68
T	2 4	2·6 8·1	— —	5·7 8·4	36 —	176 92
G	1½ 2	8·0 6·0	— —	— 5·6	— —	— —
H	2 3 4	6·8 7·1 8·6	— 3·1 —	— 7·0 —	21 — —	48 — 42
Mo	2 3	6·8 7·3	— 3·5	5·0 —	24 28	30 22
R	½ 3	5·1 —	— —	— 5·3	— —	— 83
G	1	8·0	—	5·6	—	—
			(B) Pronounced Hyperexcitability (14)			
S	2 3	4·6 6·7	— 3·6	6·8 —	18 —	44 —
L	1 2	7·3 7·8	3·6 2·9	— —	— —	46 —
W	½ 1½ 2¼	8·3 6·8 7·2	— — 4·9	4·5 — —	— — —	46 — —
D	1¼ 2	6·4 8·0	— 4·9	5·0 —	— —	32 —
N	1½ 2½	6·5 7·4	— 4·5	— 3·0	— 20	— 35
N	1½ 2½	7·3 —	4·1 —	— —	— —	— 31
T	1¾	6·1	3·2	—	—	35
T	2	8·0	—	7·1	26	62
W	1½ 2½	6·8 7·2	— 4·9	4·5 —	— —	46 —
F	2	7·6	—	—	—	—
S	1 2	5·7 6·1	— 3·2	— —	— —	35 —
Wm	1½ 2½	6·8 —	— —	— —	— —	— —
W	¾ 1¼	8·1 7·9	— —	5·4 5·0	— —	— —
McG	1¼ 2 3¼	9·0 8·0 9·8	— — —	— — —	— — —	46 — —
			(C) Mild Hyperexcitability (5)			
I	1¼	7·0	3·5	—	—	—
S	1 2	7·8 7·3	— —	5·8 —	9 —	53 —
M	2 3	5·6 7·0	2·8 —	— 7·4	— —	— 34
S	1¼	7·9	—	—	—	—
Mac	1½ 2	7·1 7·5	— 3·9	— —	20 —	35 —

* King Armstrong units.

TABLE 4

OBSTETRIC BACKGROUND IN HYPOCALCAEMIC TETANY WITHIN 36 HOURS OF BIRTH: 26 INFANTS

A. *Born at Term* (38 weeks and over)		
Healthy mother:	spontaneous delivery: breech	1
	caesarean section: prolonged labour	1
	caesarean section: placenta praevia	1
	elective caesarean section: disproportion	1
B. *Short Gestation Period* (less than 38 weeks)		
Healthy mother:	spontaneous delivery: vertex	4
	" breech	2
	both infants of a twin pregnancy	2
	one infant of twin pregnancies	2
Diabetic mother:	spontaneous delivery: vertex	3
	elective caesarean section	7
Pre-diabetic mother:	spontaneous delivery: vertex	1
Eclampsia and placenta praevia:	caesarean section	1

II. Neuromuscular Activity in the Absence of Hypocalcaemia

A tentative diagnosis of tetany based on clinical observations was made on the second day of life in seven infants in whom the total serum calcium level was above 8 mg. per 100 ml. The clinical picture was pronounced in six and mild in one. All seven babies were delivered spontaneously, presentation being by the vertex in five cases and by the breech in two. The estimated gestation period was 37 weeks or less in the case of five babies: and 40 and 41 weeks in the remaining two. In no case was there a history of maternal toxaemia. Two of the premature infants were the firstborn of twin pregnancies (see IV (2) below). The onset of neuromuscular hyperexcitability took place three, 16, 20, 23 and 29 hours after birth and persisted for 12, 30, 72, 24 and 72 hours respectively in five babies. The corresponding percentages of serum calcium on the second day of life were 8·5, 9·3, 8·8, 11·6 and 8·7 mg.

In two instances neuromuscular hyperexcitability in the absence of hypocalcaemia occurred in babies given exchange transfusions.

III. Hypocalcaemia without Associated Neuromuscular Hyperexcitability

Two examples were encountered of hypocalcaemia not associated with neuromuscular hyperexcitability. Both occurred in infants newly born to diabetic mothers. In each case the estimated gestation period was 36 weeks, delivery was spontaneous, and the serum calcium level on the second day of life was 7·3 mg. per 100 ml.

IV. Some Special Groups

The 35 infants reported in this study include a high proportion of infants born prematurely, a number of babies born to diabetic mothers, several infants of twin pregnancies and four babies given replacement transfusions on account of haemolytic disease.

(1) **Premature Infants.** The estimated gestation period of 29 babies was less than 38 weeks, ranged from 31 to 37 weeks and averaged 36 weeks. Ten of the infants were born to diabetic mothers, the average birth weight being 7 lb. 7 oz. The birth weight of one baby born to a pre-diabetic mother was 6 lb. 12 oz. The average birth weight of the remaining 18 premature infants was 4 lb. Of the 29 premature babies hypocalcaemia was a feature of 24, being associated with violent, pronounced and mild hyperexcitability in five, 13 and four cases respectively. In two babies there was no hyperexcitability despite the presence of hypocalcaemia; and hyperexcitability was present in five infants in whom the serum calcium was above 8 mg. per 100 ml.

(2) **Twin Pregnancies.** Hypocalcaemic tetany was found in the babies of four twin pregnancies. In one such pregnancy both babies were involved; and in another both infants showed clinical signs of pronounced neuromuscular hyperexcitability but only one infant had a total serum calcium level under 8 mg. per 100 ml. In each of two other pregnancies one infant showed evidence of hyperexcitability and was the only infant with hypocalcaemia. A feature of each of the four twin pregnancies was that the total serum calcium level was lower in the second than the first live-born infant. Details are given in Table 5.

TABLE 5

SERUM CALCIUM LEVELS IN FOUR TWIN PREGNANCIES

Baby	Hyperexcitability	Total Serum Calcium (mg./100 ml.)	Birth Weight (lb.)	Birth Weight (oz.)	Maturity (weeks)
N 1	Pronounced	7·4	4	6	35
N 2	Pronounced	7·3	5	7	35
T 1	Nil	8·6	5	4	36
T 2	Pronounced	5·7	3	14	36
S 1	Pronounced	8·8	3	9	34
S 2	Pronounced	7·8	3	6	34
Mc 1	Nil	8·3	3	10	33
Mc 2	Mild	7·1	3	13	33

One of the infants with pronounced neuromuscular agitation in the absence of hypocalcaemia was a twin, the other product of the pregnancy being a macerated foetus.

None of these babies was born to a diabetic mother.

(3) **Infants Born to Diabetic Mothers.** Ten babies with clinical tetany were born to mothers with diabetes mellitus and one to an obese mother with a pre-diabetic type of glucose tolerance curve. All 11 infants were premature and all were singletons. Of the 11 babies, 10 gave total serum calcium levels below 8·0 mg. per 100 ml., the values ranging from 2·6 to 7·5 mg. %. In the remaining baby the corresponding figure was 8·0 mg. per 100 ml. Two infants (see section III) with low serum calcium levels showed no clinical evidence of abnormal neuromuscular instability. They were unique in this respect among all babies included in this investigation. In babies born to diabetic mothers neuromuscular hyperexcitability was violent in four, pronounced in four and mild in two infants. Clinical signs of tetany in the baby of the pre-diabetic mother were violent.

During the period of this investigation only three babies who had been newly born to diabetic mothers were found to show neither neuromuscular hyperexcitability nor hypocalcaemia.

(4) **Exchange Transfusions.** Pronounced or violent neuromuscular hyperexcitability was encountered in four babies given exchange transfusions on account of haemolytic disease. A total serum calcium level below 8 mg. per 100 ml. was obtained in two cases. In the interval between the commencement and completion of transfusion a fall of 3 mg. per 100 ml. was recorded in one baby, and a fall of 2 mg. per 100 ml. in another. Potassium levels were not estimated. Blood used for the exchange transfusions was citrated and had been stored for a period not exceeding five days. No calcium preparations were given during the transfusions.

Discussion

Biochemical Considerations. It is known that the serum calcium level falls transitorily during the first few days of life (Editorial, 1950). Gittleman *et al.* (1956) noted that in a number of newborn infants the concentration of calcium in the serum was less than 8 mg. per 100 ml. on the first day of life. They found that the incidence of hypocalcaemia was greater in infants delivered by caesarean section and in premature infants than in babies delivered spontaneously at term. Respiratory embarrassment, a tendency to regurgitate, convulsions, tremors and twitching of the extremities were seen in a number of the premature babies, and infants delivered by caesarean section (Gittleman, 1957). Zetterström and Arnhold (1958) stress the frequent occurrence of hypocalcaemia in the newly born babies of diabetic mothers. The occurrence of 'first-day hypocalcaemia' is referred to by Gribetz (1957) and has been demonstrated by Bruck and Weintraub (1955).

Clinical Approach. The investigations carried out by Gittleman *et al.* (1956) and Bruck (1955) were concerned primarily with biochemical findings. By way of contrast the present study is based primarily upon clinical observations. Admittedly the study is of only limited scale. It can be said, however, that despite its contrasting approach the results are in many respects in keeping with those of the two American investigations. The present series provides confirmatory evidence of a noteworthy incidence of hypocalcaemia in the early hours of life more especially among premature infants, babies born to diabetic mothers, and infants delivered by caesarean section. It is of interest too that while five of the 20 premature infants with hypocalcaemia in the present series were the products of twin pregnancies the premature infants in Gittleman's series included a number of twins. Our findings further resemble those of Gittleman (1957) in that the serum calcium levels in twins of the same pregnancy were usually different.

A more important result of the present study is evidence of the presence on the first or second day of life of a characteristic clinical picture of neuromuscular excitability in a large proportion of infants in whom there is 'first-day hypocalcaemia'. The association of neuromuscular hyperexcitability with hypocalcaemia warrants use of the term tetany, and is in accordance with views expressed by Bakwin (1937, 1939), Shannon (1934), Willi (1939), Bass (1942) and Kendig (1942).

Correlation of Clinical and Biochemical Findings. In their investigation of clinical tetany appearing between the first and thirteenth day of life Dodd and Rapoport (1949) found an approximate parallelism between the severity of symptoms and the depression of calcium levels. They record that the serum calcium varied between 6 and 9 mg. per 100 ml. in the infants with clinical tetany, although in a few babies with 'mild to moderate signs' levels of above 9 mg. were obtained.

The findings in our series of infants in whom clinical signs of tetany developed within 36 hours of birth do not reveal any rigid parallelism between the severity of clinical signs and the depression of calcium concentrations. It is probably significant, however, that in clinically violent cases the total serum calcium level averaged 6 mg. per 100 ml. while the corresponding figure for all other cases with manifest signs was 7 mg. per 100 ml. This may be

an over-simplification. To some extent the physical tone and strength of an infant determine the severity of general systemic reactions to underlying trauma, infection or biochemical disturbance. This is evident in the subdued nature of the convulsions which are seen in small premature infants with severe intracranial trauma, meningitis or pyelonephritis. In the present series of infants an approximate relationship existed between birth weight and the clinical picture: the average birth weight of infants with violent, pronounced and moderate clinical tetany was 6 lb. 12 oz., 5 lb. 15 oz. and 4 lb. 10 oz. respectively. This in turn may be related to the positive correlation between birth weight of the premature infant and the concentration of calcium in the plasma noted by Gittleman and Pincus (1951).

A further point of interest is that Dodd regarded a total serum calcium level of less than 9 mg. % as evidence of hypocalcaemia in the newborn. Had this standard been applied in the present series six of the seven infants we have regarded as examples of neuromuscular hyperactivity in the absence of hypocalcaemia (section II) would have been included among babies with hypocalcaemic tetany (section I). Another consideration is the interval of time between the onset of clinical signs of tetany and the taking of specimens for calcium estimations. As already emphasized clinical signs appeared within 36 hours of birth in every baby. An endeavour was made to obtain serum on the second day of life as a routine. The need to minimize disturbance of the babies ruled out any question of subsequent daily estimates. Almost certainly therefore the lowest concentration of calcium in the serum was not determined in every case. This is of importance because we gained the impression that although an approximate parallelism existed in the individual case between the trend of the clinical pattern and that of the serum calcium level, clinical signs of tetany tended to precede the appearance of pronounced hypocalcaemia, and the gradual disappearance of clinical signs tended to lag behind elevation of the serum calcium to a normal level.

The absolute level to which the serum calcium falls may not be the only factor of significance. Severe clinical tetany developed during exchange blood transfusions in two cases in which the serum level did not fall below 8 mg. per 100 ml. The fall in level in each of these cases, however, was considerable and occurred within one and a half hours (section IV (4)). It is possible, therefore, that separately or jointly the speed and magnitude of the depression of calcium concentration may be of importance. Farquhar and Smith (1958) have investigated the disturbance of electrolyte balance which occurs during exchange transfusion. They found that the serum potassium level frequently increases and the calcium ion falls irrespective of the total serum calcium, creating conditions favouring the development of neuromuscular hyperexcitability. Estimates of serum potassium levels were not made in our study. In this study four infants given exchange transfusions, including the two referred to above, developed neuromuscular hyperexcitability in association with hypocalcaemia, and it is of interest that in three of the infants the first evidence of irritability was present before transfusion was commenced.

Differentiation of Abnormal from Normal. There is no arbitrary level of serum calcium which serves to distinguish an abnormally low from a normal concentration of calcium in the serum of the newborn infant. This applies especially to infants aged only 1 or 2 days in view of the temporary physiological fall in serum calcium level which occurs at this time. Midwives with prolonged experience of the care of premature infants are agreed that 'jumpiness', or 'preminess' as some term it, is a feature in the first few days of life of many of the smallest infants for whom they are responsible. In the present study neuromuscular hyperexcitability was noted in 27 premature babies (section IV (1)). It is probable that these examples of clinical tetany represent an intensified form of the clinical 'ditheriness' characteristic of a significant proportion of small premature babies in general. If this postulate is correct it can be argued with justification that just as no clear line of demarcation exists between physiological and pathological hypocalcaemia, so there is no definable clinical feature differentiating normal from abnormal neuromuscular excitability. This does not invalidate application of the term tetany to cases in which neuromuscular excitability and hypocalcaemia are found in association. It does, however, suggest that hypocalcaemic tetany *per se* occurring within 36 hours (say) of birth should be regarded as an exaggeration of a normal physiological state rather than as a distinct abnormal clinico-biochemical entity. The fact that unusual clinical and biochemical features disappear spontaneously gives support to this view.

It has been said that 'the concurrence of clinical tetany and hypocalcaemia [in the newborn] may suggest a causal relationship but this has not been clearly established' (Editorial, 1950). Observations made in this study do not clarify the continuing uncertainty in this connexion. They do, however, afford evidence that the occurrence of clinical tetany

within 36 hours of life is probably favoured by a number of those factors which Gittleman *et al.* (1956) found contributed to hypocalcaemia on the first day of life. These factors include premature birth, delivery by caesarean section and maternal diabetes. It is possible that multiple pregnancy may be another factor.

Summary

Hypocalcaemic tetany is defined as abnormal neuromuscular hyperexcitability associated with a total serum calcium level of 8 mg. per 100 ml. or less.

The occurrence of hypocalcaemic tetany within 40 minutes to 36 hours of birth in 26 babies is described.

'Recovery' was complete in all cases.

Details of the clinical and biochemical findings are given.

The significance of the data presented is discussed.

Generous assistance was received from many colleagues including the consultant obstetrical, resident medical and midwifery staffs of the Leeds Maternity Hospital and St. Mary's Hospital, Leeds. Particularly valuable help was given by Miss M. Pattullo, S.R.N., S.C.M., and Dr. M. Sturges. Routine laboratory examinations were undertaken by Mr. R. K. Martindale of the Leeds Maternity Hospital. Confirmatory estimates were kindly carried out under the direction of Dr. W. Goldie at St. James's Hospital, Leeds, or of Dr. W. W. Payne and Dr. R. H. Wilkinson at The Hospital for Sick Children, London. Estimates of ultrafilterable serum calcium were made at the last-named hospital. Valuable assistance in obtaining references was given by the Librarian, The Royal College of Physicians, Edinburgh.

References

Bakwin, H. (1937). *Amer. J. Dis. Child.*, **54**, 1211.
—— (1939). *J. Pediat.*, **14**, 1.
Bass, M. H. (1942). *J. Mt Sinai Hosp.*, **9**, 314.
Bruck, E. and Weintraub, D. H. (1955). *A.M.A. Amer. J. Dis. Child.*, **90**, 653.
Craig, W. S. (1958). *Pediatrics*, **22**, 297.
Dodd, K. and Rapoport, S. (1949). *Amer. J. Dis. Child.*, **78**, 537.
Editorial (1950). *Quart. Rev. Pediat.*, **5**, 271.
Farquhar, J. W. and Smith, H. (1958). *Arch. Dis. Childh.*, **33**, 142.
Gittleman, I. F. (1957). Personal communication.
—— and Pincus, J. B. (1951). *Pediatrics*, **8**, 778.
——, ——, Schmerzler, E. and Saito, M. (1956). *Ibid.*, **18**, 721.
Gribetz, D. (1957). *A.M.A. J. Dis. Child.*, **94**, 301.
Hopkins, T., Howard, J. E. and Eisenberg, H. (1952). *Bull. Johns Hopk. Hosp.*, **91**, 1.
Kendig, E. L., (1942). *J. Pediat.*, **21**, 510.
Lavietes, P. H. (1937). *J. biol. Chem.*, **120**, 267.
Mitchell, A. G. and Stevenson, F. E. (1932). *J. Amer. med. Ass.*, **99**, 1502.
Shannon, W. R. (1934). *Arch. Pediat.*, **51**, 23.
Willi, H. (1939). *Mschr. Kinderheilk.*, **80**, 309.
Zetterstrom, R. and Arnhold, R. G. (1958). *Acta paediat. (Uppsala)*, **47**, 107.

CELLULAR IMMUNITY AT BIRTH

THE MECHANISM AND NATURE OF THE PHAGOCYTIC RESPONSE

BY

M. S. EDWARDS, L. L. GRIFFITHS and P. N. SWIFT

From Farnborough Hospital, Kent

(RECEIVED FOR PUBLICATION JUNE 27, 1958)

Immunity in the infant has been extensively studied in the past and numerous investigations have been made into the development of specific immunity, the production of antibody and the passive transfer of antibody from the mother. On the other hand comparatively little work has been devoted to elucidating the complexities of the cellular response since Tunnicliff (1910) observed that phagocytosis by infants' leucocytes of streptococci, staphylococci and pneumococci was less active than in adults until the age of about 2 years. Subsequently it was demonstrated that the efficiency of phagocytosis was to a large extent determined by factors in the serum. In this connexion the studies of Mudd, Lucké, McCutcheon and Strumia (1929) were particularly important since they clarified our understanding of mechanisms by which serum enhances phagocytosis in the immunized animal and the role of antibody therein. Ward and Enders (1933) were able to elaborate upon these observations and correlated the effects of complement and antibody. They concluded that complement acts merely in the role of a catalyst which accelerates the effect of antibody. A similar view put forward by Topley and Wilson (1929) was that 'in normal serum the low concentration of specific antibodies necessitates the adjuvant action of a considerable amount of complement before its presence can be detected so that the complementary action appears to dominate the picture, while the high concentration of specific antibody in an immune serum reduces the complementary effect to a mere enhancement of an effect which takes place in its absence'.

Ward and Enders (1933), in one of the few reports dealing with infants, found that the titre of complement in infants under the age of 1 year was as high as that in adults. It seemed probable, therefore, that the inferiority of phagocytosis in infancy might be due to substances other than complement.

The findings of Gluck and Silverman (1957) are thus consistent with earlier work in that they showed that a phagocytosis-promoting factor was located in the α_1, α_2 and β-globulin fractions of the serum protein.

Matoth (1952) produced evidence which suggested that infants' leucocytes were inherently less active than those of adults and considered that the effect of serum was unimportant in spite of the fact that he noted infant serum to be less potent than adult serum in promoting phagocytosis.

The picture of cellular immunity in early infancy is thus incomplete and as much of the earlier work was carried out with immune sera and virulent micro-organisms their conclusions and results cannot be applied without due criticism to the explanation of the newborn infant's vulnerability to infections by bacteria of low pathogenicity.

The present investigation was designed in the light of previous work to study the processes involved in normal phagocytosis by the leucocytes of cord blood and the functional interdependence of leucocyte and serum in the infant at birth.

Method

The procedure for measuring phagocytosis which we adopted was a modification of that originally described by Wright (1921) for the determination of the opsonic index. The efficiency of phagocytosis is expressed as the number of leucocytes in a total count of 100 leucocytes which have ingested one or more yeast cells, as described by Hamburger (1927), and similar to the procedure employed by Mudd *et al.* (1929). We found this more reliable in practice than Wright's method in which the number of particles in each leucocyte was counted. The present method also has the advantage that when yeast cells are employed there is less difficulty in differentiating between particles within the cells and those on its surface.

Materials. Maternal blood (10 ml.) obtained by venepuncture within half an hour after delivery and 10 ml. of infants' blood from the umbilical vein was collected and 5 ml. of each placed in a dry bottle and

5 ml. into a bottle containing 50 i.u. heparin. Ten millilitres of blood from a normal adult was collected into a dry bottle for use as control.

Preparation of Sera. The serum obtained from the blood in each dry bottle after clotting and centrifugation was divided between two tubes, one of which was heated to 56° C. for 30 minutes to inactivate complement. Both were then kept at 4° C. until used, within 24 hours.

Preparation of Cell Suspensions. The plasma from 3 ml. of the heparinized maternal or infant's blood was withdrawn after centrifugation. The packed cells were then washed three times with 10 times their volume of modified Hanks' solution and the supernatant fluid withdrawn. Deposit, 0·5 ml., was aspirated from the bottom of the tube with a Pasteur pipette to effect some concentration of the leucocytes in the upper layers. The resultant leucocyte-rich deposit was kept at 4° C. until used.

Yeast Cell Suspension. Yeast cells were finally chosen since experiments with carbon particles and starch granules had proved unsatisfactory because of the difficulties and inaccuracies in counting the ingested particles. Yeast cells possess the advantage of uniform size suitable for counting and yet not so large as to cause gross distortion of the leucocyte. As Hanks (1940) demonstrated a relationship between the degree of phagocytosis and the number of micro-organisms in suspension, it has been necessary to determine the optimal concentration of yeast cells to ensure uniformity. The proportion of leucocytes exhibiting phagocytosis of yeast cells was found to increase in proportion to the number of yeast cells in suspension, until a concentration of 75,000 yeast cells per c.mm. was reached. Suspensions containing greater numbers of cells produced little further increase in the number of actively phagocytic leucocytes although the number of ingested particles per leucocyte (i.e. the phagocytic index) continued to increase. In the present experiments, therefore, a concentration of 100,000 yeast cells per c.mm. was chosen, since minor variations were found to produce no significant effect. On the other hand in estimating phagocytosis by means of the phagocytic index, variations in the number of yeast cells caused relatively large variations in the number of particles ingested. For this reason the phagocytic index was considered less reliable for studies involving numerical comparisons, though it has been estimated in the present series on the majority of specimens and shows a close correlation with the total number of active leucocytes (Fig. 1).

Preparation of Suspension of Yeast Cells. Yeast strain CN 1845 was propagated on nutrient agar and subcultured in nutrient broth for three to four days. After centrifugation and washing three times in 10 ml. normal saline the cells were suspended in normal saline at a concentration of 100,000 cells per c.mm. The selected concentration was obtained by serial dilution and counting in a Neubauer chamber. The cells in suspension were then killed by heating to 65° C. in a sealed tube for 15 minutes.

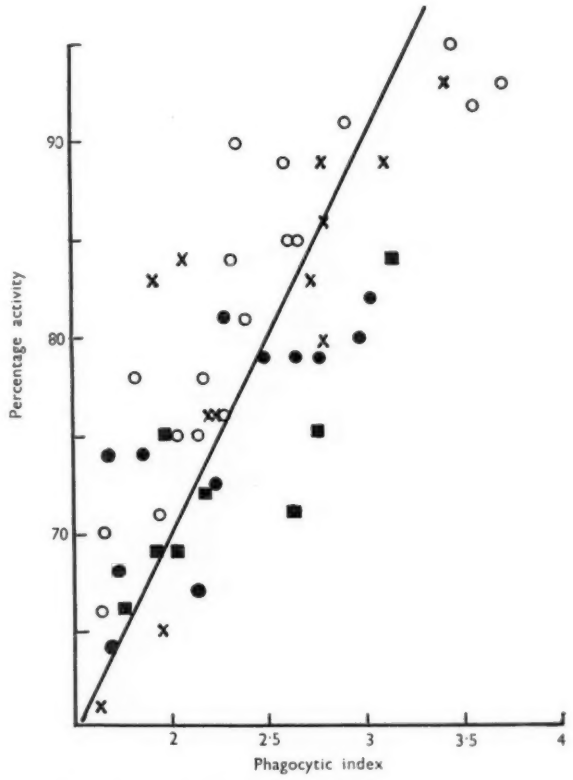


FIG. 1.—Relation of percentage activity of maternal and infant leucocytes to phagocytic index.

Demonstration of Phagocytosis. A suspension of leucocytes (0·1 ml.) of each mother or child was placed in each of six tubes containing equal volumes of serum and yeast cells, as follows:

Tube 1	Leucocytes	+ killed yeast cells			+ fresh adult serum
,, 2	,,	,,	,,	,,	+ heated adult serum
,, 3	,,	,,	,,	,,	+ fresh infant serum
,, 4	,,	,,	,,	,,	+ heated infant serum
,, 5	,,	,,	,,	,,	+ fresh maternal serum
,, 6	,,	,,	,,	,,	+ heated maternal serum

In each experiment the leucocytes of the mother and those of her own infant were prepared and tested simultaneously. After thorough mixing by shaking, the 12 tubes were placed in a water bath at 37° C. for 30 minutes and agitated every five minutes. After 30 minutes phagocytosis was inhibited by cooling in cold water, and films were made from each tube, fixed with methyl alcohol, and stained with Leishmann's stain.

Cell Counts. A total of 200 polymorphs was counted and the number which ingested one or more yeast cell noted. The number of leucocytes showing phagocytosis was expressed as percentage activity.

Twenty pairs of cell preparations in the various sera were made by this method. The mean error of the method was determined in a pilot experiment, and found to be ± 2% in counts of 200 cells.

Results

In Table 1 are tabulated the percentage numbers of leucocytes exhibiting phagocytosis of yeast cells when suspended in adult, infant and maternal sera. Comparison of the total number of active cells or the mean number of cells in each category can be made and the validity of any difference referred to Student's *t* distribution.

Comparison of the activity of the cells in their own unheated sera (columns 3 and 5A), which is the nearest approximation *in vitro* to the conditions of postnatal life, shows a small difference in mean activity between infant and maternal cells. It is not possible to make a statistical comparison under these conditions, but it is felt that the difference (79·6%-72%) may reflect somewhat greater activity on the part of maternal cells, which would be in accord with the findings of previous investigators.

The Effect of Unheated Serum on Phagocytosis. In 20 experiments the activity of infants' leucocytes has been assessed in their own unheated serum, in that of their own mother and in that of a seemingly normal adult. Likewise the activity of maternal leucocytes has been assessed in their own unheated serum, in that of their own infant and in that of an adult. The mean activity of infants' leucocytes, columns 1, 3 and 5, and maternal leucocytes columns 1A, 3A and 5A, is about the same with each of the three sera (Table 1).

Infant cells seem to be more active in maternal serum, and maternal cells more active in the sera of infants and adults, but these differences are statistically insignificant.

The Effect of Heated Serum on Phagocytosis. The influence of heated serum on phagocytosis is assessed by comparing the activity of leucocytes from an infant or mother in unheated and heated serum. The comparison has been made in their own serum, in that of their own mother or infant and in adults' serum. A highly significant difference between the number of leucocytes of infant and maternal origin showing phagocytosis in fresh and the number showing phagocytosis in heated sera is apparent (Table 1).

The influence of heated serum on phagocytosis is assessed by comparing the activity of leucocytes from the same infant or from the same mother in maternal, infant and adult sera (Table 2a, b). In each of the 20 experiments, the infants' leucocytes have been tested in their own, in their mothers' and in adults' heated serum. The maternal leucocytes have been tested in their own, their own infants' and normal adult serum.

The number of infant and maternal leucocytes exhibiting phagocytosis is significantly greater in heated maternal serum (and to a less extent heated adult serum) than in infant serum.

TABLE 1
THE NUMBER OF LEUCOCYTES % EXHIBITING PHAGOCYTOSIS

	Infant Leucocytes						Maternal Leucocytes					
	Adult		Cord Serum		Maternal		Adult		Cord Serum		Maternal	
Pair No.	1 unheated	2 heated	3 unheated	4 heated	5 unheated	6 heated	1A unheated	2A heated	3A unheated	4A heated	5A unheated	6A heated
1	58	23	62	5	60	17	65	9	64	5	70	9
2	82	30	80	6	94	65	91	24	80	10	57	15
3	68	5	57	6	75	9	88	4	86	6	86	5
4	73	19	74	4	80	10	93	10	90	6	93	10
5	73	12	66	15	69	36	80	8	77	10	82	14
6	77	8	71	7	80	9	86	12	85	8	89	12
7	87	15	80	16	81	15	89	8	87	8	92	11
8	65	10	64	11	71	23	76	12	74	5	74	10
9	64	11	66	6	64	32	68	9	71	3	65	13
10	76	14	78	8	79	19	89	4	85	9	76	11
11	75	12	76	16	68	15	85	5	92	7	81	17
12	80	22	79	15	81	40	80	12	89	5	86	11
13	82	14	84	12	82	18	83	17	91	10	83	20
14	80	4	72	3	79	9	94	8	93	7	93	7
15	73	4	69	3	67	4	70	4	75	2	61	3
16	70	14	69	14	75	21	88	14	81	10	76	14
17	69	7	66	6	70	13	74	7	75	7	84	14
18	73	19	78	15	74	18	94	13	90	9	83	13
19	84	9	75	4	79	8	90	7	95	6	89	5
20	73	10	75	5	74	7	82	5	84	4	72	9
Mean	74·1	13·1	72·05	8·85	75·1	19·4	83·25	9·6	83·2	6·85	79·6	11·15

(The numbers are the mean of two counts of 100 cells.)
In each pair the mother's leucocytes and her own infant's leucocytes are tested in their own, or the other's serum. Adult serum was obtained from a normal adult.

TABLE 2a

DIFFERENCES BETWEEN THE NUMBERS OF ACTIVE INFANT LEUCOCYTES IN THE HEATED SERA OF MOTHERS, ADULTS AND INFANTS

Heated Serum	Infant Leucocytes (% activity)	Difference (N = 20)
Infant ..	8·85	t = 2·38 (significant)
Adult ..	13·1	p ≏ 0·05
Infant ..	8·85	t = 2·99 (highly significant)
Maternal ..	19·4	p = 0·01
Adult ..	13·1	t = 1·72 (not significant)
Maternal ..	19·4	p = 0·1

TABLE 2b

DIFFERENCES BETWEEN THE NUMBERS OF ACTIVE MATERNAL LEUCOCYTES IN THE HEATED SERA OF MOTHERS, ADULTS AND INFANTS

Heated Serum	Maternal Leucocytes (% activity)	Difference (N = 20)
Infant ..	6·85	t = 2·0 (significant)
Adult ..	9·6	p = 0·05
Infant ..	6·85	t = 3·45 (highly significant)
Maternal ..	11·15	p ≏ 0·001
Adult ..	9·6	t = 1·25 (not significant)
Maternal ..	11·15	p ≏ 0·2

Inherent Activity. The intrinsic activity of infants' and mothers' leucocytes was assessed by comparing their activity when suspended in an identical serum. The results are shown in Table 3, and show that mothers' leucocytes are more active to a highly significant degree than infants' leucocytes in both infants' and adults' serum. When suspended in maternal serum on the other hand, infants' leucocytes show the same activity as those of their mothers.

Comparison of the activity of mothers' and infants' leucocytes in the same heated sera is shown in Table 4. In the heated serum of infants and adults no significant difference in their activities is demonstrable, but in heated maternal serum the infants' leucocytes show significantly more phagocytosis than their mothers' leucocytes.

Relation of Phagocytosis to Maturity of the Leucocyte. The average number of nuclear lobes per leucocyte was estimated for each infant and mother in counts of 100 cells.

In infants' leucocytes the average number of lobes was found to be 2·41 and in the mothers' leucocytes 2·86, but no relation can be demonstrated, in either unheated or heated serum, between the average number of lobes of the leucocytes and number of phagocytosing cells on the same film.

Relation of Phagocytosis to Birth Weight. The weights of the infants in the present series ranged from 6 lb. 5 oz. (2·85 Kg.) to 8 lb. 11 oz. (4·03 Kg.) at birth. All infants were delivered at full term.

No significant correlation was apparent in the weight of the infants and the activity of their leucocytes. Our findings are thus in accordance with those of Gluck and Silverman (1957).

Discussion

Previous studies on phagocytosis in infancy have established that leucocytes are less active at this age than in later childhood and adult life. Matoth (1952), who employed starch granules, concluded that the leucocytes were themselves relatively inactive and considered that the influence of substances in the serum was small. Gluck and Silverman (1957), however, have demonstrated that the addition of adult serum to the blood of babies of birth weight below 2,000 g. brings about a significant increase in the proportion of effective phagocytes.

It seems probable in view of these reports that the inherent activity of the cells and the enhancing effects of serum may both contribute to the inferiority of infant leucocytes.

The results of the present investigation confirm the existence of significant differences between the phagocytic activity of cord and mothers' leucocytes under certain conditions.

The Phagocytosis-promoting Factors in Serum. Although infants' and mothers' leucocytes appear (Table 3) to be less active in their own unheated serum than in heterogeneous serum the difference is small and may well have occurred by chance. The figures therefore indicate that phagocytosis occurs with similar frequency in both unheated sera and no significant difference between the effects on phagocytosis by infants' and mothers' serum can be assumed.

TABLE 3

DIFFERENCES BETWEEN THE ACTIVITIES OF INFANT AND MATERNAL LEUCOCYTES IN UNHEATED SERA

Serum	Infant Leucocytes (% activity)	Maternal Leucocytes (% activity)	Difference (N = 20)
Infant ..	72·05	83·2	t = 4·8 (highly significant)
Adult ..	74·1	83·2	t = 2·9 (highly significant)
Maternal	75·1	79·6	t = 1·0 (not significant)

TABLE 4

DIFFERENCES BETWEEN THE ACTIVITIES OF INFANT AND MATERNAL LEUCOCYTES IN HEATED SERA

Serum	Infant Leucocytes (% activity)	Maternal Leucocytes (% activity)	Differences (N = 20)
Infant ..	8·85	6·85	t = 1·5 (not significant)
Adult ..	13·1	9·6	t = 1·9 (not significant)
Maternal	19·4	11·15	t = 2·4 (significant)

For this reason the seeming superiority of maternal over infant leucocytes, albeit small, cannot simply be attributed to differences in whole serum on the strength of these findings.

Phagocytosis by infant and maternal leucocytes of particles sensitized by heated serum is much less efficient than that of particles sensitized by unheated serum. As a result of the greater loss in the activity of mothers' leucocytes in heated serum, the activity of infants' cells is equal to or greater than that of their mothers. We have no evidence that this is due to a greater capacity for spontaneous phagocytosis on the part of infant leucocytes.

The influence of the different sera on phagocytosis has been studied in order to determine whether this finding might be explained by differences in the sera.

In contrast to the constancy with which ingestion occurs in unheated sera, well-marked and significant variations are observed in heated sera (Table 2a, b). In the heated serum of infants, phagocytosis by infants' or mothers' leucocytes is less than that in heated adult serum and less to a highly significant degree than that in heated maternal serum. These observations imply that serum factors determine activity in the absence of complement, and they suggest, moreover, that the heat-stable enhancing factor is of importance in determining the ultimate activity of the phagocyte. We may assume that the heat stable fraction of serum which enhances phagocytosis is present with greatest effect in maternal serum and least effect in infant serum. Whether this is due to quantitative or qualitative differences in the active constituents of the heat-stable fraction is not known, though the findings of Gluck and Silverman (1957) and our own studies on the serum globulin are compatible with the view that they may be due to qualitative changes. In the present investigation the Popper reaction, which depends on the amount of α and β globulins, was higher in heated serum of most infants (mean 3·85 compared with 2·65 in unheated serum) and of all mothers (mean 4·3 compared with 2·3 in unheated serum). The Kunkel reaction, which depends principally upon the γ-globulin fraction, was reduced in all heated sera and especially in maternal sera (mean 1·25 compared with 4·3 in unheated serum). In order to confirm these changes electrophoretic separation (Griffiths, 1952, 1953; Griffiths and Brews, 1953) was performed on seven paired sera and it demonstrated that a small increase in the amount of α_2-globulin and a small reduction in the γ-globulin occurs as a result of heating maternal serum.

The influence of the heat-labile fraction of serum may be estimated by finding the numerical difference between activity in unheated and in heated serum. The figure thus obtained is a measure of the number of leucocytes which owe their activity to the heat-labile fraction. It offers a convenient means of measuring quantitatively the phagocytosis-promoting effect of heat-labile fraction or complement.

When this figure is plotted graphically against the number of active cells in unheated serum (Fig. 2a, b) a close correlation with the overall activity of

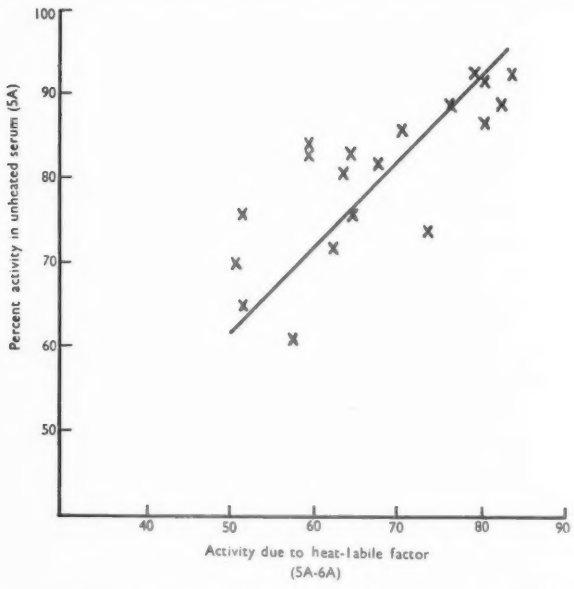


FIG. 2a.—Relation of activity of maternal leucocytes in unheated maternal serum (5A) to activity due to heat-labile factor (5A-6A).

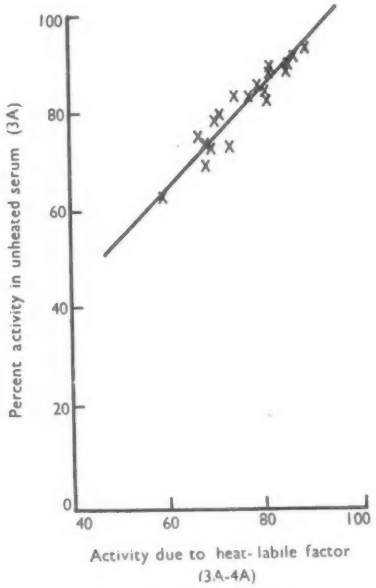


FIG. 2b.—Relation of activity of maternal leucocytes in unheated infant serum (3A) to activity due to heat-labile factor (3A-4A).

maternal leucocytes is demonstrated, which indicates that phagocytosis is directly related to the opsonic effect of the heat-labile fraction of serum.

This finding is in accordance with present views on the role of complement as the dominant influence on normal phagocytosis.

Since the correlation of total phagocytosis by maternal leucocytes and heat-labile fraction is the same in both infant and maternal serum it may be inferred that complement is present in infant serum to the same extent as in maternal serum, as found by Ward and Enders (1933). Since we have been unable to demonstrate any lack of effective complement in infant serum, differences in phagocytic activity between infant and maternal leucocytes cannot be explained by postulating differences in complement. In so far as it is possible to separate the functions of leucocyte and serum it appears that the heat-stable fraction of infant serum in some way as yet undetermined is deficient in a phagocytosis-promoting factor. Of the substances in serum which are not destroyed at 56° C. specific antibodies are known to influence phagocytosis. We have attempted by employing a non-pathogenic strain of yeast throughout these experiments to avoid interference by specific immunity and we consider such interference most improbable.

Hormonal status particularly in respect of oestrogens and corticosteroids influences phagocytosis, but the concentration of these substances in the blood of mother and infant is similar at birth, and differences in phagocytosis would not be expected unless their leucocytes differ in sensitivity to the effects of hormones.

The Response of the Leucocyte. From the foregoing discussion upon the phagocytosis-promoting effects of serum it is apparent that the paucity of an active heat-stable factor in infant serum accounts at least in part for the relative inactivity of infant leucocytes. This conclusion is not inconsistent with the conclusions of Gluck and Silverman (1957) that the activating component may be in the α and β fractions of the serum protein, though proof that the factor observed by us is identical with that of Gluck and Silverman is not within the scope of the present investigation.

The observation that the infants' leucocyte is relatively more dependent upon a heat-stable factor than upon complement suggests that there may be a difference in the cells' response to sensitized particles. In this connexion it is interesting to note that the heat-stable phagocytosis-promoting factor in maternal serum does not exert an effect of similar magnitude on the leucocytes of the mother herself (Table 4) which seems to be affected mainly by complement (Fig. 2).

Evidence of the nature of the differences between the responses of maternal and infant leucocytes can be obtained by further analysis of their behaviour under certain conditions.

It has already been shown that phagocytosis by maternal leucocytes in unheated serum bears a close quantitative relationship to activity due to complement and is thus presumed to be determined by complement (Fig. 2). The activity of infant leucocytes bears some but not such a close relation to complement in their own serum, and in maternal serum no relation that can be demonstrated (Fig. 3a, b, c).

Since it has already been shown that the opsonic effect of complement is similar in both sera it is concluded that the avidity of infants' leucocytes for particles sensitized in the presence of complement is less than that of their mothers'.

In maternal serum, in spite of a striking absence of any quantitative relationship to complement, the total activity of infant cells is greater. The knowledge that maternal serum is comparatively rich in a heat-stable enhancing factor combined with the knowledge that the activity of infants' leucocytes is not quantitatively dependent on complement provides evidence that their activity is more dependent on a heat-stable factor in serum. The fact that the mothers' leucocytes retain their dependence upon complement excludes the possibility that this is a non-specific effect of maternal serum and serves to confirm that there is an inherent difference in cellular response. Likewise our findings are not compatible with the view that this is an effect of antibody passively transferred from the mother.

The difference in response is characterized by a marked avidity of infants' leucocytes for particles sensitized by the heat-stable fraction of maternal serum. In nature, however, there are no opportunities after birth for micro-organisms invading the infant to be sensitized by maternal serum and thus for the infant to receive any protection which this mechanism might provide. If it does play a role in defence against infection in life it must do so while the foetus is *in utero* and the placenta intact, for only in these circumstances are conditions such that invading micro-organisms will have been sensitized by contact with maternal serum. As there is no significant difference between the activity of mothers' and infants' leucocytes when there has been sensitization by unheated maternal serum (Table 3), it is probable that, *in utero*, the foetal leucocytes are as efficient as those of their mothers.

The present investigation has shown that the

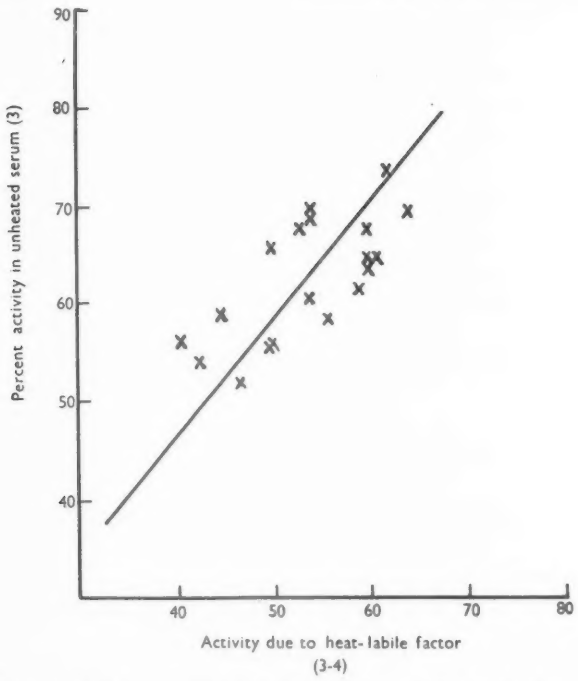


FIG. 3a.—Relation of activity of infant leucocytes in unheated infant serum (3) to activity due to heat-labile factor (3-4).

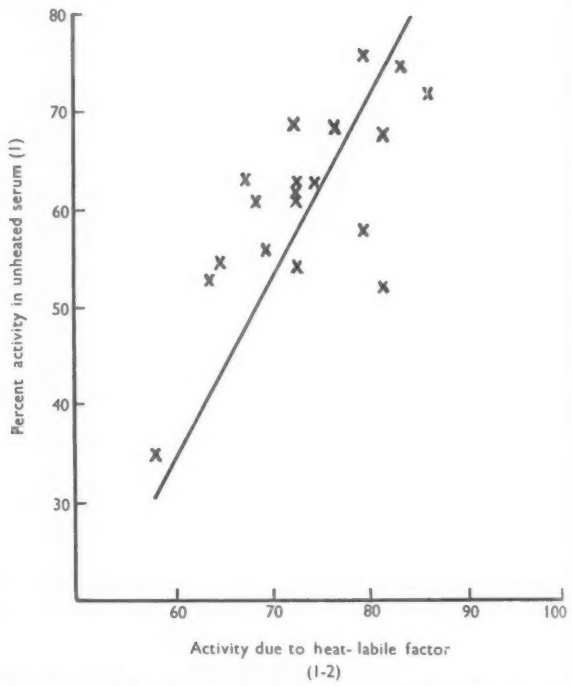


FIG. 3b.—Relation of activity of infant leucocytes in unheated adult serum (1) to activity due to heat-labile factor (1-2).

cellular response in the normal non-immune infant at birth resembles the response in the immune adult, in which antibody appears to be relatively more important than complement. The evidence suggests that this altered or perhaps more primitive response is due to an inherent difference in the leucocyte at this age. It differs from that in the mother by virtue of differences in their serum which is relatively deficient in a heat-stable enhancing factor and by virtue of differences in the leucocytes which appear to be more avid for yeast cells sensitized by the heat-stable fraction of maternal serum and less to the effects of complement.

Conclusion

The results of the present investigation provide evidence of differences between the mechanisms controlling phagocytosis in cord blood and those in maternal blood. Normal phagocytosis by maternal leucocytes is determined primarily by the heat-labile fraction of serum. Normal phagocytosis by leucocytes of cord blood, on the other hand, is less responsive to the effect of heat-labile factors and more to the effect of heat-stable factors in serum. Although observations on the nature of the heat-stable factor have been made, its identification is outside the scope of the present investigation.

This heat-stable factor is found in cord, adult and

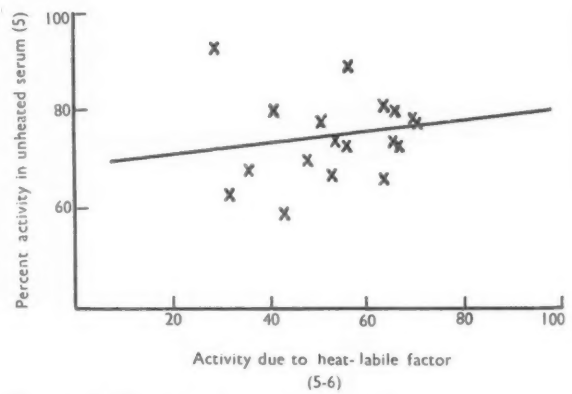


FIG. 3c.—Relation of activity of infant leucocytes in unheated maternal serum (5) to activity due to heat-labile factor (5-6).

maternal sera, but to greatest effect in maternal serum, and to least effect in cord serum. The leucocytes of cord blood have a relatively greater avidity for particles sensitized by maternal serum.

While it is possible that these phenomena may have some protective role in the foetus, it is difficult to visualize how they might benefit the infant after birth. The withdrawal of protection afforded by maternal serum and the readjustment of the cellular response to one dominated by complement may well play a part in the infant's vulnerability to infection.

Our findings are not compatible with views which attempt to explain the infant's susceptibility on the basis of simple and separate defects in its leucocytes or serum. The functions of leucocytes and serum are closely interdependent and in the infant at birth both show significant differences from those of the mother.

Summary

The phagocytic activity of leucocytes has been measured by finding the percentage which ingest killed yeast cells. Activity as estimated by this method bears a close correlation to the phagocytic index.

The activity of the leucocytes of cord blood and of the leucocytes of the mother have been measured in unheated cord, maternal and normal adult serum and in heated cord, maternal and adult serum.

The efficiency of phagocytosis by cord and mothers' leucocytes in different sera can be compared to demonstrate the influence of serum and leucocytes on phagocytosis.

Unheated cord and maternal serum are equally effective in promoting phagocytosis.

Heated cord serum is significantly less effective than heated maternal serum in promoting phagocytosis.

Heated sera are much less effective than unheated sera in promoting phagocytosis.

Mothers' leucocytes are more active than those of their infants except in mothers' unheated serum when activity is equal.

In heated sera, infants' leucocytes are as active as those of their mothers except in mothers' heated serum where they are more active.

The activity of maternal leucocytes is closely correlated with the heat-labile fraction of serum which is present in both infants' and mothers' sera. The activity of infants' leucocytes is less closely dependent on the heat-labile factor of serum and especially of mothers' serum.

It is concluded that cord serum is relatively deficient in a heat-stable factor capable of enhancing phagocytosis and that the leucocytes of cord blood are less dependent upon the heat-labile factors in serum than are maternal leucocytes. The heat-stable fraction of maternal serum most effectively enhances phagocytosis by cord leucocytes.

To Dr. S. R. M. Bushby we are indebted for his assistance in selecting the test organism and for supplies of yeast strain CN 1845. We wish to thank both Dr. Bushby and Professor George Brownlee for their advice and helpful criticisms at all stages of the work. The study was made possible by a financial grant received from the South-east Metropolitan Regional Hospital Board.

References

Gluck, L. and Silverman, W. A. (1957). *Pediatrics*, **20**, 951.
Griffiths, L. L. (1952). *J. clin. Path.*, **5**, 294.
—— (1953) *J. Lab. clin. Med.*, **41**, 188.
—— and Brews, V. A. L. (1953). *J. clin. Path.* **6**, 187.
Hamburger, H. J. (1927). In *Handbuch der biologischen Arbeitsmethoden*, ed. E. Abderhalden. Abt. 4, teil 4, p. 953. Urban and Schwarzenberg, Berlin.
Hanks, J. H. (1940). *J. Immunol*, **38**, 159.
Matoth, Y. (1952). *Pediatrics*, **9**, 748.
Mudd, S., Lucké, B., McCutcheon, M. and Strumia, M. (1929). *J. exp. Med.*, **49**, 779.
Topley, W. W. C. and Wilson, G. S. (1929). *The Principles of Bacteriology and Immunity*, Vol. 1, p. 176. Arnold, London.
Tunnicliff, R. (1910). *J. infect. Dis.*, **7**, 698.
Ward, H. K. and Enders, J. F. (1933). *J. exp. Med.*, **57**, 527.
Wright, A. E. (1921). *Technique of the Teat and Capillary Glass Tube*. Constable, London.

THROMBOTIC MICROANGIOPATHIC HAEMOLYTIC ANAEMIA

BY

M. J. ROBINSON, N. P. ORCHARD and A. R. EDEN

From the Austin Hospital, Heidelberg, Melbourne

(RECEIVED FOR PUBLICATION JUNE 9, 1958)

Introduction

The syndrome of thrombotic microangiopathic haemolytic anaemia is rare and almost all the cases reported have been in adults. Very few cases occurring in childhood have been reported and the youngest recorded case was 22 months old (Craig and Gitlin, 1957).

We report the clinical and pathological details and the response to treatment of a child aged 21 months, who was seen at the Austin Hospital, Melbourne.

Case Report

J.L., a male child aged 21 months, was admitted to the Austin Hospital, Melbourne, with a week's history of malaise, anorexia, fever and a suggestion of pain, tenderness and mild swelling of both wrists and ankles. Six days before admission a red, blotchy urticarial rash was noted over the diaper area but this faded after three days.

He had had similar but milder symptoms following immunization with triple antigen, also following a course of sulphonamide, and one mild attack appeared spontaneously. These early episodes lasted only a few days. There were no significant past illnesses and the family history was not relevant.

On physical examination he was flushed and uncooperative. The temperature was 103° F., the pulse 164 and respirations 28 per minute. A fine purpuric rash diffusely involved the face and both upper extremities. Both wrists were swollen and tender and movement was restricted by pain; in addition, there was mild swelling of both knees and ankles. A mild tonsillitis and an otitis media were present. A provisional diagnosis of anaphylactoid purpura of the Schönlein type was made and he was treated with aspirin.

During the first week in hospital his temperature ranged between 99° F. and 103° F. (Fig. 1). The blood examination showed the haemoglobin to be 9 g. % (60%), the white cell count 20,000 per c.mm., with 70% neutrophils (showing a marked shift to the left), 25% lymphocytes and 5% monocytes; the red cells and platelets were normal, and no lupus erythematosus cells (repeated examinations), glandular fever cells or malarial parasites were seen; the reticulocyte count was less than 1%. Examination of the sternal marrow revealed no abnormality. The clotting and prothrombin times were normal but clot retraction was poor. The Paul-Bunnell and Coombs tests were negative. Repeated blood cultures and agglutination tests, the Mantoux and Casoni tests and the Wassermann and Kahn reactions were all

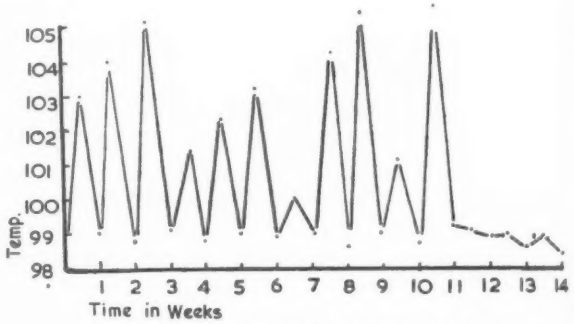


FIG. 1.—Temperature range during first admission.

negative. Liver function tests, the blood urea and the serum proteins and electrophoresis were normal. The cerebrospinal fluid was normal as were radiographs of the chest and long bones. The throat swab grew a β haemolytic streptococcus.

The rash reappeared during the second week and the temperature, malaise and irritability persisted. A four-week course of aureomycin did not affect the temperature.

One month after admission the spleen became palpable and there was a mild generalized lymphadenopathy. At this stage the white blood count rose to 32,400 per c.mm. but the haemoglobin did not fall. After three months of hospitalization his temperature subsided spontaneously and being quite well he was allowed to go home. He remained well for the next six months when he developed a rash, resembling erythema marginata, on his arms and chest. Six weeks later the swinging temperature recurred and on one day his joints became painful. Similar episodes recurred weekly until he was readmitted three weeks later because of persistent pyrexia, irritability and a disclination to move his limbs.

Examination at this stage revealed a fine generalized red papular rash, slight generalized lymphadenopathy and a temperature of 103° F. (Fig. 2.) The haemoglobin was

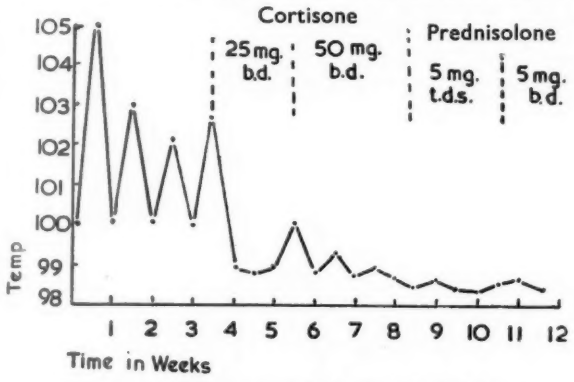


FIG. 2.—Temperature range during second admission.

11 g. % (74%); white cell count 24,600 per c.mm. with 60% neutrophils, 4% eosinophils, 33% lymphocytes and 3% monocytes; reticulocytes 12%. The platelets were 100,000 per c.mm.; this was the lowest figure obtained, and the count the next day was normal. In addition the red cell fragility in hypotonic saline was slightly increased and spherocytes were observed for the first time. The serum bilirubin was 3·2 mg./100 ml. and urinary urobilinogen was markedly increased and the faecal urobilinogen excretion per day was 300 mg.

The white blood count gradually rose to 56,000 per c.mm. in the next four weeks; the marked shift to the left and the presence of 8% myelocytes rendered the blood picture leukaemoid. The haemoglobin level waxed and waned in association with a fall and rise in the reticulocyte count, which reached 15% at one stage. The spherocytes increased in number and became a permanent feature.

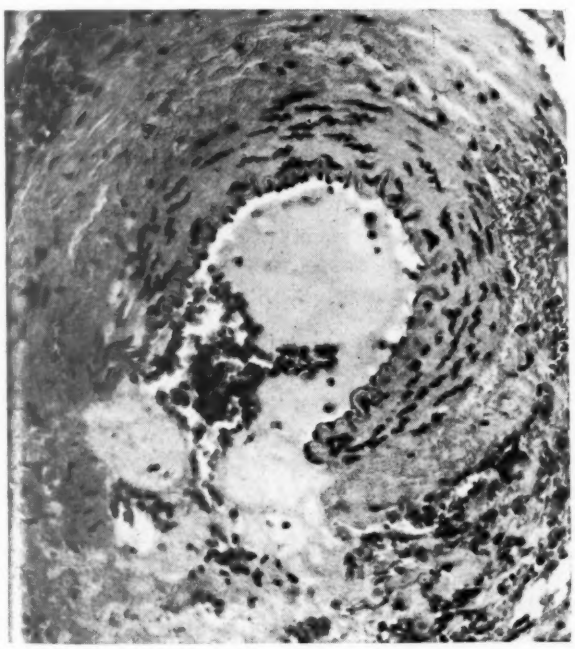


FIG. 3.—Section of the arteriole in the pericapsular region of the lymph node, showing a hyaline thrombus, rupture of the vessel wall and haemorrhage into the surrounding tissues. Haematoxylin and eosin. × 700.

The possibility of thrombotic microangiopathic haemolytic anaemia was suggested by one of us (N.P.O.) and a combined axillary lymph node and pectoral muscle biopsy was taken. Sections of the muscle showed no abnormality. Sections of the lymph node revealed reactive hyperplasia only, but striking changes were observed in the small calibre vessels in the pericapsular region (Fig. 3.) One vessel was almost completely occluded by a mass of hyaline material. There was a breach in the vessel wall with resulting haemorrhage into the perivascular tissues. There was no perivascular reaction whatsoever. Other vessels were occluded and in the thrombi could be seen endothelial cells, fibroblasts and the clefts of recanalization. Some vessels showed endothelial hyperplasia and others subendothelial accumulation of hyaline material. These histological findings confirmed the diagnosis. Sections were stained by Weigert's, Mallory's P.T.A.H., Biebrich scarlet-aniline blue and Pearse's P.A.S.-orange G-haematoxylin stains, and most of the thrombi were partially positive for fibrin.

Cortisone therapy was started, with initially 25 mg. of cortisone acetate twice daily by mouth. The improvement was dramatic; his temperature subsided in 10 hours and although occasionally elevated in the next two weeks this was never above 100° F. When the dose was increased to 50 mg. b.d. the temperature remained completely normal. Equally dramatic was the clinical improvement; his personality completely changed, he became co-operative, his appetite returned and he immediately wanted to get out of his cot. Before this he was quite miserable and inconsolable. With 50 mg. b.d. he developed features of Cushing's syndrome and prednisolone 5 mg. t.d.s. and later 5 mg. b.d. was substituted for cortisone acetate. Since that time he has required between 5 and 10 mg. of prednisolone daily; the mother has now learnt from his general demeanour and from his temperature chart (which she has kept assiduously) how much cortisone is required. The constant aim is to stop cortisone therapy but at this stage a remission has not been obtained. Clinically, however, he is very well indeed and physical examination reveals no abnormality.

Discussion

The first example of this syndrome was described by Moschcowitz (1924, 1925). Probably no more than 40 proven cases have been reported since. According to Symmers and Barrowcliff (1951) this syndrome occurs in both sexes at any age. The youngest case recorded so far was a child, aged 22 months, reported by Craig and Gitlin (1957). All cases to date have died, two-thirds of them in six weeks or less. Examples of a prolonged course are few, the longest being that of a young man reported by Brown and Norman (1946). Another

case running a course of three and a quarter years was reported by Meacham, Orbison, Steele and Schaefer (1951).

The onset of the syndrome is usually sudden. Typically it is characterized by fever, purpura and haemolytic anaemia. Multifarious, fluctuating neurological disturbances often occur terminally and are of little diagnostic consequence, and death results, usually in two to four weeks.

Abnormal laboratory findings include thrombocytopenia and anaemia often associated with a raised reticulocyte count. Leucocytosis is usual and may be leukaemoid in type. The bleeding time is prolonged and clot retraction defective but the coagulation time is usually normal.

The case is of interest for several reasons: it is the youngest one on record, the mode of presentation although acute was by no means fulminating and the illness has been protracted. Frank neurological signs have been absent from the start, although there was a marked personality change consisting of extreme misery and complete lack of co-operation even after months in hospital. With a fall in the temperature the patient became quite quickly a happy and co-operative child. On one day only was the platelet count down to 100,000 per c.mm., but this was normal the next day and subsequently. Anaemia was never at any time severe, the lowest haemoglobin level recorded being 9 g. % (60%).

The clinical presentation with fever, leucocytosis, marked joint symptoms, an urticarial rash and a mild purpura, has been recorded before.

Evidence of hypersensitivity, as illustrated here, to pertussis-diphtheria-tetanus antigen and sulphonamides is not infrequent in this syndrome and reactions to iodine compounds, sulphonamides, and penicillin have been reported. Many consider this syndrome to be related to the so-called collagen diseases. We felt that the symptomatology was consistent with that of disseminated lupus erythematosus, but L.E. cells were not demonstrated. There is a good deal of pathological evidence to support this view. Verrucous endocarditis and disseminated lupus erythematosus have been reported in association with thrombotic microangiopathy. Symmers and Gillett (1951) have also reported a case of polyarteritis nodosa in which widely disseminated lesions of thrombotic microangiopathy were also present. Beigelman's (1951) concept of the aetiology of disseminated lupus erythematosus, polyarteritis nodosa, and thrombotic microangiopathy is that of a hyperergic response occurring at different sites in the vessel wall; a maximal response in the perivascular mesenchyme produces disseminated lupus erythematosus, a maximal response in the media produces polyarteritis nodosa, and a maximal response in the intima produces thrombotic microangiopathy. Similar lesions can be reproduced in animals by the Schwartzman phenomenon.

The thrombotic lesions, which are confined to the smallest calibre vessels, occur more frequently and are more numerous in the myocardium, the capsular zones of the adrenals and in the renal cortex. The hepatic and adrenal sinusoids are not involved. There has been, in the past, much speculation as to the exact nature of the occlusive thrombi. Although it was generally accepted that these thrombi were composed of agglutinated platelets, there was no definite proof. Craig and Gitlin (1957) reviewed the literature at length and discussed the inadequacy of the standard staining procedures for fibrin. Using fluorescein-labelled rabbit anti-human fibrin serum, they showed that the thrombi were composed of fibrin. Also with this fluorescent antibody technique, they failed to demonstrate platelets in the thrombi.

The names given to this syndrome in the past have been unsatisfactory. While some have stressed the thrombocytic nature of the thrombi and others the thrombocytopenia, they all lose sight of the characteristic haemolytic anaemia. Moschcowitz's syndrome was suggested as a title, but being eponymous it is not entirely satisfactory. Symmers (1952) suggested the name of thrombotic microangiopathic haemolytic anaemia or, for short, thrombotic microangiopathy, which is descriptively accurate and wisely omits controversial and inconstant features. It is to be hoped that this title finds general acceptance.

The prognosis in the present case must obviously be guarded. Cases living longer than this one have been reported but have invariably terminated fatally. However, the response to steroid therapy is the encouraging feature of this case. The recorded cases have almost always been so acute that therapy has not been successful. A.C.T.H. and cortisone have been used and splenectomy has been performed but without benefit. We feel that in this case cortisone proved of decided value in inducing the second remission. Reduction of prednisolone below 5 mg. b.d. results in elevation of the temperature, malaise and joint soreness within three days. We have persistently tried to discontinue therapy but the reappearance of pyrexia and symptoms has been prompt.

Summary

The youngest case (21 months) of thrombotic microangiopathic haemolytic anaemia is recorded. The child is alive and well after two years.

The clinical and pathological findings are given in detail. The nature of the occlusive thrombi is discussed.

The names accorded this syndrome are discussed and the title, thrombotic microangiopathic haemolytic anaemia, or for short, thrombotic microangiopathy, is supported.

Steroid therapy has been of inestimable benefit and no doubt has been life-saving. As yet it has not been possible to discontinue the maintenance dose of prednisolone. The prognosis must obviously remain guarded.

References

Beigelman, P. M. (1951). *A.M.A. Arch. Path.*, **51**, 213.
Brown, E. B. and Norman, J. W. (1946). *N.Y. St. J. Med.*, **46**, 2167
Craig, J. M. and Gitlin, D. (1957). *Amer. J. Path.*, 33, 251.
Meacham, G. C., Orbison, J. L., Steele, H. J. and Schaefer, J. A. (1951). *Blood*, **6**, 706.
Moschcowitz, E. (1924). *Proc. N.Y. path. Soc.*, **24**, 21
—— (1925). *Arch. intern. Med.*, **36**, 89.
Symmers, W St. C. (1952). *Brit. med. J.*, **2**, 897.
—— and Barrowcliff, D. F (1951). *J. Path. Bact.*, **63**, 552.
—— and Gillett, R. (1951). *A.M.A. Arch. Path.*, **52**, 489.

ACUTE ACQUIRED HAEMOLYTIC ANAEMIA ASSOCIATED WITH HERPES SIMPLEX INFECTION

BY

R. McLAREN TODD and N. V. O'DONOHOE

From Alder Hey Children's Hospital, Liverpool

(RECEIVED FOR PUBLICATION JUNE 13, 1958)

Acquired haemolytic anaemia in childhood is a relatively uncommon condition. Lederer (1925) described three patients with acute haemolytic anaemia and in the literature up to 1938 Atkinson (1940) found 59 cases of 'Lederer's anaemia', 30 of which were in children. Millichap (1952) reviewed the papers published between 1940 and 1951 and found reports of a further 32 children with this disorder, the youngest patient affected being 6 weeks of age.

Acquired haemolytic anaemia may develop after treatment with drugs such as quinine, sulphonamides, para-amino-salicylic acid, or after chemical poisons, for example naphthalene, benzene; it may be associated with general diseases such as leukaemia, lymphadenoma, liver diseases, lupus erythematosus; it may follow or be associated with infections either caused by bacteria (*Streptococcus*; *Cl. welchii*; *Cl. tetanis*) or by viruses (virus pneumonia, *influenza A*, *coxsackie A*, infectious mononucleosis, Newcastle disease, measles); but it is frequently unassociated with any obvious disease although investigations may point to the presence of auto-agglutinins or auto-haemolysins. Boorman, Dodd and Loutit (1946) considered that congenital and acquired haemolytic anaemias could be distinguished by the antiglobulin (Coombs) test which was thought to be positive only in acquired haemolytic anaemias; but Wright, Dodd, Bouroncle, Doan and Zollinger (1951) have shown that the Coombs test may be negative in acquired haemolytic anaemia and positive in the congenital type. The stimulus to production of the antibodies in this disorder remains a mystery in many cases but the most likely explanation is that the antigen is derived from infective agents such as bacteria or viruses. The clinical features and results of investigations in a patient recently under our care are now described.

Case Report

Ann O'D., aged 6 years 3 months, was admitted to Alder Hey Children's Hospital on February 2, 1957. Two months before admission she had a clinically mild attack of measles and no complications developed. She returned to school and remained well until five days before admission when she was off colour and did not want to eat. Her family doctor found clinical evidence of a throat infection and prescribed oral penicillin. Her condition did not improve with this therapy and her parents noticed increasing pallor. On the day before admission she vomited once and gradually became semi-comatose. No bleeding was observed from the gastro-intestinal tract or elsewhere.

Her mother, father, sister and three brothers were healthy and there was no family history of allergy, tuberculosis or other disease. When admitted to hospital she was in a semi-comatose condition, extremely pale and with a slight icteric tinge. Oral temperature was 99·8 F., pulse rate 160 per minute, respirations 25 per minute, and sighing in character. No petechiae were present in the skin or mucous membranes, but a few bruises ('normal') were present on both shins. Oral examination showed that the gums were healthy but a few herpetic lesions were present on the inside of the cheeks. There were no signs of infection in the throat, ears, or lungs and no lymphatic glandular enlargement in the neck, axillae or groins. The heart was not enlarged but generalized haemic murmurs were heard, and the systolic blood pressure was 80 mm. Hg. The liver was palpable one finger breadth below the costal margin and the spleen was enlarged to two finger breadths below the costal margin. The optic fundi and general neurological examination did not reveal any abnormal signs.

The haemoglobin was 14% (2·1 g. %); white cells 2,600 per c.mm (neutrophils 58%, lymphocytes 18%, monocytes 15%); the red cells showed spherocytosis and the platelets were present in normal numbers. Direct Coombs test was strongly positive. Total serum protein level was 5·9 g. %, albumin 2·49 g. (42·3%), globulin 3·41 g. (57·7%), A/G ratio 0·73 to 1; γ globulin 1·50 g. % (25·5%), β_2 0·21 g. % (3·6%), β_1 0·39 g. (6·6%), α_2 0·75 g. % (12·6 %) and α_1 0·56 g. % (9·4%). These results show a γ globulin level twice the normal, a slightly raised α_1, and a lowered β_1. Paul Bunnell reaction was negative. Blood transfusion was started on admission, and a total of two pints of packed red cells and one pint of blood was given over a period of 26 hours.

A C.T.H. was given intramuscularly in a dosage of 80 units twice daily for five days, followed by prednisolone 15 mg. three times daily for five days, reducing by 5 mg. every two days and discontinued four weeks after admission. Progress has been satisfactory and hepatosplenomegaly gradually subsided over a period of 10 months. Table 1 shows the results of serial blood investigations.

TABLE 1
SERIAL BLOOD INVESTIGATIONS

Date	Hb (g. %)	Hb (%)	W.B.C. (per c.mm.)	Reticulocytes (%)	Direct Coombs
2.2.57	2·1	14	2,600		Strongly positive
6	9·2	62			Positive
12	8·9	60	11,000		Positive
20					Positive
25	11·8	80		12	
6.3.57	13·3	90		2·3	Negative
14	13·3	90			
28	10·4	70			
11.4.57	11·8	80			Positive
16	12·3	83		1·3	
9.5.57	12·3	83	9,000	7·1	Positive
23	11·5	78	7,000		Positive
20.6.57	10·4	70			Positive
25.7.57	10·9	74			Negative
5.9.57	11·7	79			Negative
29.11.57	11·4	77			Positive
11.3.58					Positive

Virus studies gave the following results:

Complement fixation tests negative for mumps and L.C.M. positive for recent herpes simplex infection.
Day 4 1 in 20
„ 20 1 in 320
Neutralization tests herpes simplex
Day 4 positive 1 in 16
negative 1 in 32
„ 20 positive 1 in 256
(only taken to this dilution)

We failed to isolate any agent from this child's blood by inoculation of chorio-allantoic membrane of fertile hen's eggs and passage of these membranes after three days' incubation of primary eggs at 36° C. Serum still had a titre of greater than 1 in 256 in the egg neutralization test.

During the past five years, four other patients with acute acquired haemolytic anaemia have been admitted to Alder Hey Children's Hospital, and Table 2 summarizes the relevant features.

Discussion

The onset of acute acquired haemolytic anaemia is often dramatic and treatment by blood transfusion to restore the haemoglobin level to a more normal value is an urgent matter. In our patient, the initial haemoglobin reading was 14% and in the four other patients seen in this hospital the haemoglobin levels reached 17%, 22%, 31% and 40% (Table 2); in Millichap's (1952) five patients haemoglobin levels varied from 18% to 48%, and in the four patients reported by Rose and Nabarro (1953) haemoglobin levels varied from 12% to 28%. A satisfactory response to blood transfusion is usually as dramatic as the onset of the illness but sometimes transfusion makes the situation worse because the transfused blood is haemolyzed rapidly. In these circumstances a replacement transfusion has proved of considerable value (Bowman, 1955), for this procedure removes antibody attached to the red cells and free in the plasma, and the fresh blood dilutes any free antibody that might remain in the circulating blood. Among the 21 children suffering from acute haemolytic anaemia reported between 1940 and 1950 (Millichap, 1952) 18 (85%) responded to blood transfusions alone. Steroid therapy is a useful adjunct to blood transfusion and probably acts by interrupting the antigen-antibody reaction, thus leading to a fall of antibody and hence to a lessening of the haemolytic process. In idiopathic acquired haemolytic anaemia, the basic disorder appears to be the patient's ability to form antibodies which have an affinity for the red blood cells. It is thought that antibodies are contained in the γ globulin fraction of the plasma and it is of interest that in our patient the γ globulin level was twice the normal value. These antibodies are usually pan-antibodies, i.e. they attack all human red cells, irrespective of blood group but elution of the red cells of our patient yielded anti-e. Weiner, Battey, Cleghorn, Marson and Meynell (1953) have described a man of 32 years with acute haemolytic anaemia in whom the antibody was also anti-e. Dacie (1954) has found that auto-antibodies in idiopathic haemolytic anaemia are of two main kinds, warm and cold. He reported the presence of warm antibodies in 30 patients and cold antibodies in eight patients with acute haemolytic anaemia; in seven of these

TABLE 2
FEATURES OF FOUR PATIENTS WITH ACUTE ACQUIRED HAEMOLYTIC ANAEMIA

Case	Age	Duration of Symptoms Before Admission	Hb (%) On Admission	Hb (%) Lowest Level	Plasma Methaemalbumin	Direct Coombs	Coombs Test Negative After:
J. V. ..	6 m.	6 d.	22	22	+	−	*
H. V. ..	1 yr. 7 m.	6 w.	40	40	+	+	18 m.
K. McD.	2 yr. 2 m.	3 w.	48	31	+	+	8 w.
E. ..	12 yr.	2 w.	52	17	−	+	6 w.

* Died after 5 days. Post mortem examination showed renal cortical necrosis.

the illness followed virus pneumonia and in one measles preceded the anaemia.

Infections often precede the development of these acute anaemias and it is reasonable to suppose that the blood in its circulation through virus-infected tissue becomes contaminated with virus particles which are adsorbed on to the red cell surfaces; these virus-contaminated red blood cells may stimulate the reticulo-endothelial system to produce antibodies which destroy the red blood cells and also lead to a rising blood antibody titre. In eight patients with idiopathic haemolytic anaemia reported by Morgan (1955), tests for Newcastle disease virus, influenza, mumps, psittacosis, herpes and pox virus were negative; in the four children reported by Rose and Nabarro (1953) tests for influenza, glandular fever and psittacosis were negative. Moolten and Clark (1952) isolated Newcastle disease virus from the blood of a baby of 11 months and a patient of 37 years suffering from acute idiopathic haemolytic anaemia, and they also found an unidentified virus in the blood of 10 patients with acute haemolytic anaemia associated with general diseases such as leukaemia, Hodgkin's disease, lupus erythematosus, and infective hepatitis. Although we were unable to isolate a virus from the blood of our patient, the results of complement fixation and neutralization tests confirmed that a recent primary infection with herpes simplex virus had occurred. McNair Scott (personal communication) does not know of a report of a similar case, and we have been unable to find a similar example of the development of haemolytic anaemia following herpes simplex infection in the British or American literature.

Summary

The clinical features and results of haematological investigations of a girl of 6 years suffering from acute acquired haemolytic anaemia are recorded. Investigations suggest that the anaemia was the result of a recent primary infection with herpes simplex virus.

References

Atkinson, F. R. B. (1940). *Brit. J. Child Dis.*, **37**, 35.
Boorman, K. E., Dodd, B. E. and Loutit, J. F. (1946). *Lancet*, **1**, 812.
Bowman, J. M. (1955). *A.M.A. Amer J. Dis. Child.*, **89**, 226.
Dacie, J. V. (1954). *Sang*, **25**, 675.
Lederer, M. (1925). *Amer. J. med. Sci.*, **170**, 500.
McNair Scott, T. F. (1958). Personal communication.
Millichap, J. G. (1952). *Arch. Dis. Childh.*, **27**, 222.
Moolten, S. E. and Clark, E. (1952). *Trans. N.Y. Acad. Sci.*, **14**, 232.
Morgan, H. R. (1955). *J. Lab. clin. Med.*, **46**, 580
Rose, B. S. and Nabarro, S. N. (1953). *Arch. Dis. Childh.*, **28**, 87.
Wright, C. S., Dodd, M. C., Bouroncle, B. E., Doan, C. A. and Zollinger, R. M. (1951). *J. Lab. clin. Med.*, **37**, 165.
Weiner, W., Battey, D. A., Cleghorn, T. E., Marson, F. G. W. and Meynell, M. J. (1953). *Brit. med. J.* **2**, 125.

HAEMOLYTIC ANAEMIA IN A CHILD RECEIVING NITROFURANTOIN

BY

DAVID LEVY and ALICE PORGESS

From the Paediatric Department, Poriah Government Hospital, Tiberias, Israel

(RECEIVED FOR PUBLICATION MAY 28, 1958)

In 1956, West and Zimmerman reported a 'first case' of haemolytic anaemia, apparently caused by the use of nitrofurantoin (Furadantin). Since then, no further reports of this untoward effect of the drug have appeared in the literature.

Recently a case of acute pyelonephritis treated with Furadantin, observed in the Paediatric Department of the Poriah Government Hospital in Tiberias, developed severe haemolytic anaemia when given this drug.

Case Report

S.I., a male baby of Iraqui origin, was admitted to this department at the age of 10 months. He was born normally, after a normal full-term pregnancy, the eighth child in the family. He had been breast fed all his life. No haemolytic disease was reported among the other children or the parents of the baby.

A week before admission the infant was seen by a local physician on account of watery stools and vomiting. The treatment given was, reportedly, not effective.

On admission, the baby was severely ill. He was semi-conscious; breathing was deep and rapid, giving the impression of severe metabolic acidosis. Skin turgor was practically non-existent. The liver edge was felt 3 cm. below the costal margin. The temperature was 39° C., and weight 7·3 kg.

Laboratory examinations on admission showed the following results: Hb. 11·0 g. per 100 ml.; R.B.C. 3,900,000 per c.mm.; W.B.C. 41,000 per c.mm. (stab 7%, neutrophils 68%, lymphocytes 18%, monocytes 1%, eosinophils 1%, plasma cells 2%); reticulocytes 3%. Repeated urine examinations showed albumin +, white blood cells in large clumps and many granular casts. Stool examination was negative; stool culture was sterile for *Shigella* and *Salmonella*. Old tuberculin (5 TU) intracutaneously caused no local reaction 48 hours after injection.

It was apparent that this baby was suffering from dehydration and electrolyte imbalance; he was therefore given intravenously physiological solutions with added base. During the 24 hours after admission 600 ml. of standard solution, saline and glucose 5% aa, 400 ml. of 5% glucose solution and 50 ml. of 1/6 molar sodium lactate were administered. The baby was also given Neomycin 400 mg. daily, in four equal doses.

On the day after admission the baby's condition was markedly improved; body weight reached 8·1 kg.; the number of stools was reduced and no clinical signs of acidosis were apparent. On the fourth day of hospitalization stools were normal in appearance and number.

On the third day of his hospital stay, because of the urinary tract infection, a course of nitrofurantoin, 50 mg., three times a day, was started. On the fourth day of this treatment he passed red dark-coloured urine. Administration of nitrofurantoin was discontinued, after a total dose of 700 mg. had been given, a total of 90 mg. per kg. of body weight.

On this day the red blood cell count was 980,000 c.mm. with a haemoglobin level of 3 g. per 100 ml. The reticulocyte count was 8%, platelets 140,000 per c.mm.; haematocrit 17%. The total serum bilirubin level was 2·82 mg. per 100 ml.; urea 38·5 mg. per 100 ml. The total plasma protein was 5·8 g. per 100 ml. (3·08 albumen and 2·72 globulin). A Coombs test, direct and indirect, was completely negative. Auto-antibodies at 36° C. and at 6° C. were not found. No autohaemolysins and isohaemolysins were discovered after adding complement. Bone-marrow examination showed increased erythropoiesis with an M:E ratio of 1·7:1. Paper electrophoresis of haemoglobin showed haemoglobin A only. By alkali denaturation 3·1% foetal haemoglobin was found (normal for the patient's age). There was no bile in the urine, but haemoglobin was present.

The baby was given 450 ml. of blood over a six-hour period. Haemoglobin went up to 7·4 g. per 100 ml., the red blood cell count to 2,810,000 per c.mm. A week later further regeneration brought the red blood cell count to 3,200,000 per c.mm. and the haemoglobin rose to 9·8 g. per 100 ml. The total serum bilirubin level fell to 1 mg. per 100 ml. with a direct fraction of 0·6 mg.

Treatment was continued with Achromycin 300 mg. per day. The baby made an unhampered recovery from the urinary tract infection and was discharged after 28 days of hospitalization.

Comment

The minor side effects of nitrofurantoin are well known and the clinical picture is generally familiar to physicians. Nausea, vomiting, skin rashes, headache, diarrhoea and dizziness have been described

occasionally. These effects are purported to subside rapidly when the drug is withdrawn or the dosage reduced.

Rare cases of more severe toxic effects have also been reported. Thus Hasen and Moore (1954) have reported a reduced white blood cell count of 2,900 per c.mm. with a normal differential count in one of 68 patients treated with Furadantin. West and Zimmerman (1956) described the appearance in a negro male of haemolytic anaemia following the administration of nitrofurantoin. Whereas haematological and blood chemistry examinations seemed to provide good grounds for concluding that the haemolytic anaemia was related to the administration of nitrofurantoin in this patient, the authors properly reserved judgment as proof was not unequivocal and no other reports of this untoward reaction have been published.

Therefore the appearance of haemoglobinuria, reticulocytosis, a drastic fall in the circulating red blood cells and haemoglobin and a rise in serum bilirubin in a patient with pyelonephritis when treated with Furadantin caused profound interest and every effort was made to exclude other possible causes for haemolytic anaemia at our patient's age. On the other hand no trial was made to prove experimentally *in vivo* the haemolytic influence of nitrofurantoin because of the rapid haemolysis which necessitated an immediate transfusion of blood.

The mechanism of haemolysis due to nitrofurantoin is conjectural. The similarity of the nitrofurantoin-induced haemolysis to that induced by other drugs, such as the sulphonamides, is apparent. Szeinberg, Sheba, Hirshorn and Bodonyi (1957) have postulated that a constitutional congenital anomaly, namely glutathione deficiency of the red blood cells, is essential for the pathogenesis of this reaction. Their study indicates that this abnormality is fairly frequent among Jews originating from the Arab countries in Asia (Eastern Jews). No examination of the glutathione content of the red blood cells of this patient was made, as the deficiency appears with the ageing of the red blood cells, the young ones being normal.

Summary

A case of acute haemolytic anaemia occurred in an infant receiving nitrofurantoin (Furadantin) for acute pyelonephritis. No other cause for this haemolysis could be found. Haemolysis subsided after stopping the drug. Although no experimental proof is available, it seems reasonably proved that the haemolytic anaemia was caused by the action of Furadantin on the red blood cells.

REFERENCES

Hasen, H. B. and Moore, T. D. (1954). *J. Amer. med. Ass* , **155**, 1470.
Szeinberg, A., Sheba, C., Hirshorn, N. and Bodonyi, E. (1957). *Blood*, **12**, 603.
West, M. and Zimmerman, H. J. (1956). *J. Amer. med. Ass.*, **162**, 637.

ERYTHROCYTE SEDIMENTATION RATE, C-REACTIVE PROTEIN DETERMINATION, SERUM PROTEIN FRACTIONS, FIBRINOGEN LEVEL AND ANTI-STREPTOLYSIN REACTION IN 52 RHEUMATIC CHILDREN

BY

N. POPOV and S. STANISHEVA

From the Department of Rheumatism, Second City Children's Hospital, Sofia

(RECEIVED FOR PUBLICATION JUNE 16, 1958)

This paper reports the results of our investigations into the clinical significance of laboratory tests in 52 cases of rheumatic fever treated in the Rheumatic Department of the Second City Children's Hospital, Sofia. The diagnosis of active rheumatic infection is based on the history, presence of carditis (in 48 cases), changes in the electrocardiograms, and by the supporting evidence of five laboratory tests: namely the erythrocyte sedimentation rate, C-reactive protein determinations, serum protein fractions, the blood fibrinogen level and the antistreptolysin-O reaction.

Eighteen of the children were in their first attack, and 34 had had one or more attacks of rheumatic fever. Thirteen cases in first attacks and 19 cases in subsequent attacks received steroid therapy.

Five children were 6 years old or under, 36 were between 7 and 11 years, and 11 were 12 or 13 years of age.

Methods

The laboratory tests were carried out by the following methods:

1. **Erythrocyte Sedimentation Rate** (E.S.R.). By Westergren's method (1921, 1957), estimations up to 10 mm. in the first hour being accepted as normal.

2. **C-reactive Protein** (C.R.P.). Determined by the method of Anderson and McCarty (1950), using C.R.P. anti-serum produced by Schiffelin and Co., New York, and assessed as −, +, ++ or +++, in accordance with the criteria suggested by Wurhmann and Wunderly (1957).

3. **Serum Protein Fractions** (S.P.F.). By paper electrophoresis following Flynn and de Mayo (1951). Figures for each fraction expressed as percentage of the total protein, and the following figures accepted as pathological: albumen under 47%, α_1 globulin over 5%, α_2 globulin over 12%, β globulin over 14%, and γ globulin over 20% (Popov, 1956a, b). In the present paper the determinations for total protein are not discussed since deviations from the normal were slight and indeterminate.

4. **Plasma Fibrinogen Level.** By kjeldahlometric determination, normal range was 180-350 mg. per 100 ml. in accordance with our own findings (Popov and Stanisheva, 1958).

5. **Antistreptolysin Reaction** (A.S.O.). By the method described by Kalbak (1947). Standard serum from the State Serum Institute, Copenhagen, was used. Though a titre of over 200 was accepted as pathologically increased, we have previously shown (Popov and Bojadzieva, 1957) that serial estimations have a very much greater value than a single initial estimation.

In all a total of 1,743 estimations were carried out on the 52 children as follows:

E.S.R.	527 estimations
C.R.P., S.P.F. and A.S.O. ..	304 estimations each
Plasma fibrinogen	304 estimations

Results

Table 2 presents the results obtained for the five investigations on the 52 children, divided firstly into those in a first attack and those in subsequent attacks, and also divided according to whether hormone therapy was given or withheld. With each estimation the highest figure obtained during the active phase of the infection is given. Consequently for the E.S.R., C.R.P. and the fibrinogen levels, the results are obtained mainly at the beginning of the illness. For the A.S.O. the results obtained are usually in the middle of an attack, whilst the figures for the S.P.F. were obtained at various times throughout the attack.

Consideration of these results reveals the following points:

1. **Erythrocyte Sedimentation Rate.** The E.S.R. remained normal in seven children, of whom only one was in a first attack. Very high figures were obtained in 18 children, 10 in their first attack.

TABLE 2

	No.	E.S.R. (mm./1st hr.)				C.R.P.				S.P.F.				Fibrinogen Level (mg.%)			A.S.O. (units)		
											Increased								
		>10	11 to 30	31 to 60	Over 60	–	+	++	+++	Normal	α_1 α_2 γ	α_2 γ	γ only	180 to 350	360 to 550	over 550	under 200	300 to 600	300 and over
I. First attack (18)																			
(*a*) with steroid therapy ..	13	–	2	2	9	1	–	4	8	1	8	4	–	5	3	5	1	5	7
(*b*) without steroid therapy	5	1	1	2	1	–	2	–	3	–	4	1	–	2	3	–	–	2	3
II. Subsequent Attacks (34)																			
(*a*) with steroid therapy ..	19	2	5	6	6	–	2	10	7	–	9	7	3	10	6	3	3	7	9
(*b*) without steroid therapy	15	4	4	5	2	4	7	1	3	4	1	5	5	12	2	1	8	5	2
Totals ..	52	7	12	15	18	5	11	15	21	5	22	17	8	29	14	9	12	19	21

The follow up of the E.S.R. showed that in the children in their first attack and receiving steroids it remained raised for an average of 25 days, whilst in three of these the E.S.R. was not normal until the thirtieth day, and in another not until the forty-fifth day. Of the five children in their first attack not given steroid therapy, in one the E.S.R. was normal, in two it became normal in about 20 days, whilst in the remaining two, both of whom suffered from tonsillitis during their rheumatic infection, the E.S.R. remained raised for 60 days.

Of the children in a subsequent attack and receiving steroid therapy, 11 showed a raised E.S.R. for an average of 50 days (in two children for 65 days, in one child for about three months); of the children in a subsequent attack and not receiving steroid therapy the E.S.R. became normal also on an average in about 50 days (in two children after two months and in one after three months).

2. C.R.P. In general the C.R.P. tests showed positive or negative results much in accordance with the data quoted in the literature (Müller, 1956), but in many cases variations in the intensity of the reaction were obtained especially from + to ++ during the course of the disease. Of 18 children in the first attack 11 showed a raised C.R.P., usually ++. In one child the estimation was persistently negative in spite of clinical evidence of active infection, whilst in six children estimations varied from + to ++ from time to time.

Of 35 children with subsequent attacks, in 10 the C.R.P. was +++ steadily over a long period, in four it was constantly negative. Of the eight children in the first attack and being treated with hormones, C.R.P. tests in seven steadily improved from the onset of the disease, and in one (a protracted case with several previous attacks) the C.R.P. remained positive for about three months as did the E.S.R. and S.P.F. In general this test was usually the first to appear positive and the first to become negative with the exception of the fibrinogen level which was often the first to become negative in children with a first attack receiving steroid therapy.

3. S.P.F. Protein fractions are related to the humeral changes in the connective tissue during the different phases in rheumatic progress (Chaptal, Jean, Campo, Alram and Fraisse, 1954). In the present series also the dysproteinaemia was present at the beginning of the attack and its intensity appeared to correspond to the degree of the exudative and productive tissue changes. Moreover, the dysproteinaemia remained raised for a long time after the E.S.R. and S.R.P. had become negative. These findings are in accordance with those of Jacox (1956).

In the present series, five children showed repeatedly normal values in spite of other evidence of activity, four of whom were in subsequent attacks and not receiving steroid therapy. In the remaining 47 dysproteinaemia was marked at the beginning of the attack. In 22, there was at the beginning of the attack an increased α_1 and α_2 globulin with a moderately increased γ globulin. As the process evolved the α globulin fractions decreased and the γ globulin fraction increased up to a figure of 30%. In this group the dysproteinaemia lasted for an average of 55 days. By contrast, of the 17 children in their first attack, though there was a similar pattern for the α_2 and the γ fractions, the dysproteinaemia lasted on an average about 40 days. In two children who had an intercurrent infection of measles γ globulin dropped to 10 and 12% respectively.

We agree with the data of Payne and Forsyth (1958) that children over the age of six years have approximately adult protein fraction levels. We also agree with Klaishevitsch (1958) regarding the behaviour of the globulin fractions in the cardiac forms of rheumatism and that in a first attack

great deviations from the normal are observed, also a more rapid return to normal compared with children in subsequent attacks.

4. Fibrinogen Levels. We previously studied these in 70 children (Popov and Stanisheva, 1958). It was there concluded that during the first attack without complications and also in many repeated attacks the fibrinogen level may become normal during the first week of the disease, but that if the condition becomes protracted, and particularly in repeated attacks, the fibrinogen level remains high for a long time. In the present series, in 29 children fibrinogen levels were normal, between 180 and 350 mg. %; of 23 children with high fibrinogen levels, and in three cases with previous attacks and receiving hormone treatment the fibrinogen levels remained high for 30, 40 and 60 days respectively. In the remaining cases there was a greatly raised fibrinogen level, from 360 to 850 mg. %, which became normal during the first 10 days.

5. A.S.O. Twelve of our children had a titre constantly under 200 units, i.e. within the upper limit of normal, and 11 of these had had previous attacks. In 19 the A.S.O. titre was between 300 and 600 units; seven of these were in their first attack, and 12 had had previous attacks. In 21 patients the A.S.O. titre was between 800 and 1,600 and 10 of these were in their first attack. In children with a raised A.S.O. titre the height of the curve was reached about the end of the second week, whilst in those treated with cortisone the high level was sustained for from 30 to 75 days. Two children were exceptional in that the A.S.O. fell before the twentieth day. Chaptal *et al.* (1954) and Harris, Needleman, Harris and Friedman (1956) and Lanza, Morbidelli and Papotti (1956) reported that a quick fall of the A.S.O. was brought about by steroid therapy. There were three such cases in our series receiving steroids. In general, in children with previous attacks and treated with steroids the A.S.O. level remained high for 40 days.

In eight patients there was a very high level of A.S.O. titre at the beginning of their admission to hospital. In these children there was a history suggesting that they had been admitted during the second or third week of their illness.

In nine children receiving steroid therapy, three of whom were in their first attack, when steroid therapy was discontinued or diminished there was a simultaneous increase of the E.S.R., C.R.P., S.P.F., and some tendency to elevation of the A.S.O., as also clinical deterioration of the cardiac condition. This rebound phenomenon is in accordance with the findings of Holt (1956).

An effort was made to correlate the laboratory findings of the cases given steroids with the following results: 23 cases showed a correlation between the five tests, in nine cases, a correlation between the E.S.R., C.R.P., S.P.F. and the A.S.O.; in 10 cases between the E.S.R., the C.R.P., and the S.P.F.; in four cases between the C.R.P., the S.P.F. and the A.S.O., and in three cases between the C.R.P. and the A.S.O. Completely normal laboratory findings in the presence of clinical evidence of activity was observed in three cases.

Summary

At intervals of seven to 10 days 1,743 investigations for E.S.R., C.R.P., S.P.F. and fibrinogen levels and A.S.O. titre were performed in 52 rheumatic children with active infection. Eighteen of these children were in their first attack, of whom 13 were being treated with steroid therapy, and 34 had had previous attacks, 19 of whom were receiving steroids.

In 23 cases there was good correlation between the positive findings in the five tests when related to clinical activity. In three cases the laboratory tests were normal in the face of evidence of clinical activity. In the remaining cases there was correlation between four tests in nine cases, between three tests in 14 cases, between two tests in three cases. There was a rebound phenomenon in nine cases.

References

Anderson, H. C. and McCarty, M. (1950). *Amer. J. Med.*, **8**, 445.
Chaptal, J., Jean, R., Campo, C., Alram, D. and Fraisse, J. (1954). *Arch. franç. Pédiat.*, **11**, 1055.
Flynn, F. V. and Mayo, P. de (1951). *Lancet*, **2**, 235.
Harris, T. N., Needleman, H. L., Harris, S. and Friedman, S. (1956). *Pediatrics*, **17**, 29.
Holt, K. S. (1956). *Arch. Dis. Childh.*, **31**, 444.
Jacox, R. F. (1956). *N.Y. St. J. Med.*, **56**, 672: (cf. *Abstr. Wld Med.* (1956). **20**, 215.)
Kalbak, K. (1947). *The Antistreptolysin Reaction.* State Serum Institute, Copenhagen.
Klaishevitsch, G. I. (1958). *Pediatrija*, no. 1, p. 68.
Lanza, I., Morbidelli, R. and Papotti, G. (1956). *Minerva med.* (*Torino*), (*parte Sci*), **47** (1), 553.
Müller, W. (1956). *Z. Rheumaforsch.*, **15**, 31.
Payne, W. W. and Forsyth, C. C. (1958). *Arch Dis. Childh.*, **33**, 61.
Popov, N. (1956a). *Sovr. Med.*, **7**, 116.
—— (1956b). Scientific Works of the State Institute for Haematology and Blood Transfusion in Sofia, **3**, 64.
—— and Bojadzieva, M. (1957). *Sovr. Med.*, **8**, 33.
—— and Stanisheva, S. (1958). *Pediat. Akuš. Ginec.*, **2**, 29.
Westergren, A. (1921). *Acta med. scand.*, **54**, 247.
—— (1957). *Triangle*, **3**, 20.
Wuhrmann, F. and Wunderly, C. (1957). *Die Bluteiweisskörper des Menschen*, 3rd ed. Basel.

THE PROGNOSIS OF IDIOPATHIC RENAL ACIDOSIS IN INFANCY WITH OBSERVATIONS ON URINE ACIDIFICATION AND AMMONIA PRODUCTION IN CHILDREN

BY

ELIZABETH U. BUCHANAN and G. M. KOMROWER

From the Royal Manchester Children's Hospital and the Department of Paediatrics and Child Health, University of Manchester

(RECEIVED FOR PUBLICATION APRIL 30, 1958)

Idiopathic renal acidosis of infancy has been recognized for over 20 years since the descriptions of the morbid anatomy by Lightwood (1935) and of the clinical condition by Butler, Wilson and Farber (1936). The incidence of the condition appeared to reach a peak between 1946 and 1952 during which time 35 cases were described in The Hospital for Sick Children, London (Lightwood, Payne and Black, 1953), while 17 cases were seen in the Birmingham Children's Hospital between 1948 and 1953 (Carré, Wood and Smallwood, 1954). The prognosis has been stated to be good in infants surviving the acute phase of the illness (Hartmann, 1939; Stapleton, 1949; Lightwood *et al.*, 1953) but only isolated cases have been followed for any number of years after recovery (Kelsey, Reinhart and Fishel, 1950; Doxiadis, 1952). Our colleagues, seeing cases of renal tubular acidosis in adults, have been interested to know whether there is any relationship between this condition and that seen in infancy. It was, therefore, felt worth while to make a detailed survey of the eight children who had been in the Royal Manchester Children's Hospital with infantile renal acidosis between 1948 and 1953 and who, by the time of the review, were between 5 and 10 years of age and had been off all treatment for periods ranging from three and a half to nine years.

The review which was carried out in 1957 included recent history, physical examination, a radiograph of the abdomen to detect renal calcification, and examination of blood and urine. Distal renal tubular function was examined by a test designed to assess powers of concentration and dilution at the same time as the ability to secrete an acid urine and to form ammonia following the administration of ammonium chloride. We could find no data on normal values in children for urine acidification and ammonia production following oral ammonium chloride, so it was necessary to carry out observations on a control series.

Methods

Twenty-two normal children formed the control series. In selecting cases for this series the following criteria were observed: the child was considered to have recovered from the condition for which he had been admitted to hospital and was about to be discharged (the majority being surgical cases on the day before discharge); secondly, there was no history of renal disease, no abnormality was detected in the urine and the concentration and dilution test was normal.

The technique used for the combined concentration and dilution and acidification test was as follows. Fluids were restricted from 8.30 p.m. on the evening preceding the test until 12.30 p.m. next day, but solid foods were allowed. The child emptied his bladder at 8.30 p.m., 12.30 a.m. and 6.30 a.m.; the specific gravity was measured and the urine discarded. At 8.30 a.m. and 9.30 a.m. the child again emptied his bladder; the volume and specific gravity of the urine were measured and a specimen preserved with thymol for estimation of pH and ammonium content. Between 9.30 a.m. and 10.30 a.m. the child was given ammonium chloride in a dose of 0·1 g. per Kg. body weight (the salt being given in gelatine capsules in every instance except one where difficulty was encountered with swallowing, and a mixture was given instead). During that hour the child was also given 20 ml. water per Kg. body weight. The ammonium chloride was given over an hour to avoid nausea, and difficulty in this respect arose in only one instance. The child having the test emptied his bladder at 11.30 a.m. and this specimen was discarded. This procedure was followed to eliminate the fluctuations in ammonia production during this period resulting from the variable initial urine pH. In this way it was possible to assess more accurately the effect of the ammonium chloride. The bladder was emptied at 12.30 p.m., 2.30 p.m. and 4.30 p.m.; urine passed between these times

was mixed with the next timed specimen; all collections of urine were preserved with thymol. The volume and specific gravity of each specimen was measured and pH and ammonium content estimated. The pH was measured by means of the B.D.H. capillator. Ammonium was estimated using Conway's micro-diffusion units, the rate of excretion in micro-equivalents per minute being calculated in each period and also over the two to seven-hour period following the administration of the drug.

In every case the urine pH fell below 5·3 within five hours of the administration of ammonium chloride, the range being from 4·8 to 5·2 on the B.D.H. capillator. The values obtained by this means are only accurate to within 0·2 in either direction. This has been determined by comparison with values obtained on a glass electrode pH meter. It has been found, however, that when the pH is below 5·7 the figures obtained with the B.D.H. capillator is consistently higher than that obtained with the glass electrode pH meter. It seems likely, therefore, that the ability of these children to acidify urine is even greater than that shown by our results.

The rate of ammonium excretion in the five-hour period under investigation varied considerably from one child to another but was found to bear a close relationship to the child's weight. The results obtained in the 22 normal children are shown on the accompanying graph which also includes figures for normal adult controls obtained during a similar investigation in adults. In this investigation ammonium excretion was estimated over the two to eight-hour period after ammonium chloride (Wrong and Davies, 1959, in press).

Cases Under Review

The eight children who had been in the Royal Manchester Children's Hospital with infantile renal acidosis between 1948 and 1953 had all been under the care of one of us (G.M.K.): five were boys and three girls. All had been admitted to hospital between the ages of 5 and 11 months, their symptoms having begun at between 3½ and 8 months of age. With one exception they had presented three or more of the recognized features of constipation, vomiting, anorexia, failure to gain weight and polyuria. In the exception the only presenting feature was failure to gain weight. Physical examination on admission had shown two or more of the recognized signs of hypotonia, wasting, dehydration, malar flush and palpable faeces in the abdomen. In every case except one (R.M., see Appendix) the diagnosis was confirmed shortly after admission by the finding of a systemic acidosis with serum bicarbonate of less than 35 vol. % (16 mEq./l.) associated with alkaline or faintly acid urine, the pH ranging from 6·9 to 7·9. The serum chloride was raised above 600 mg. % as sodium chloride (110 mEq./l.) in the five cases in which the estimation was carried out before treatment was begun. With one exception (R.M.) the blood urea did not rise above 50 mg. % and returned rapidly to normal when treatment was begun. Radiographs of the abdomen showed no evidence of renal calcification in any patient. In seven cases (excluding R.M.) treatment with alkalis was given, sodium citrate and citric acid being used initially and potassium citrate added later if necessary. Hartmann's solution and M/6 lactate were used in the early stages of treatment especially in the four cases where parenteral fluids were required. Treatment was continued until the biochemistry remained normal after a trial period without alkali. The length of treatment varied from five to 23 months. All the children except one were able to stop treatment between the age of 15 and 21 months. The one exception was an infant who had been very difficult to control when treatment was initiated. In her case treatment had to be continued until she was 2 years 4 months old. All the children attended the out-patient department at intervals until 1954 and their progress was considered to be satisfactory.

The review carried out in 1957 comprised two stages. In the first the children were seen as out-patients and the object of the review explained to the parents. The recent history was obtained, a physical examination carried out and a radiograph of the abdomen taken. In the second stage the child was admitted to hospital for 36 hours for examination of blood and urine, followed by the combined concentration, dilution and acidification test already described.

In every case the parents were well pleased with the child's progress and no abnormality was found on physical examination which included blood pressure estimation. Heights were close to the 50 percentile for the corresponding age, but weights were rather below it with two girls close to the 10 percentile. The percentiles used were those calculated by Tanner (1958) from data provided by Scott (1955). It is interesting, though probably not significant, that the two underweight girls were the patients whom it had been most difficult to control at the onset of treatment. A radiograph of the abdomen revealed no evidence of renal calcification in any of the children. Urine examination, including microscopy, showed no abnormality. Blood chemistry comprised serum calcium, phosphorus, chloride and bicarbonate and blood urea. The values obtained were normal in every case with the exception of the blood urea in R.M. (see Appendix).

The test designed to assess distal renal tubular function showed very satisfactory results. After 13 hours' deprivation of fluid the urine specific gravity rose to between 1,018 and 1,025, while after

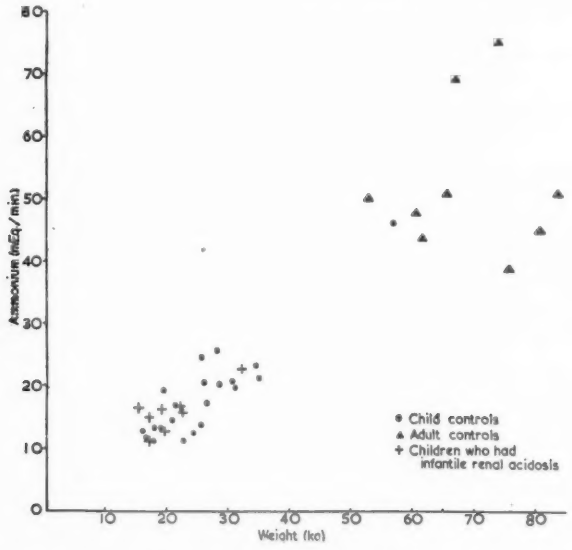


FIG. 1.—Ammonium excretion in 2-7 hours following the administration of ammonium chloride.

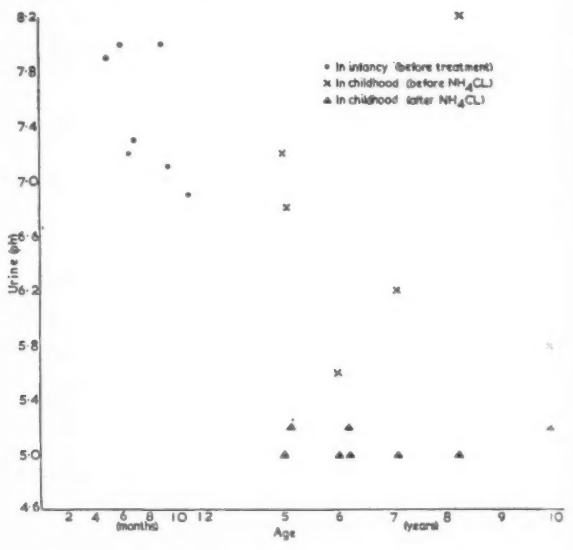


FIG. 2.—Urine pH in relation to age.

taking water (20 ml. per kg.) it fell to 1,001 or 1,000. Following ammonium chloride the urine pH fell to between 5·0 and 5·2 in every case and, bearing in mind the remarks made in connexion with the control series, the actual acidity of the urine may well have been greater. Ammonium excretion is plotted against weight in Fig. 1 and shows that these children fall within the range determined for the control series.

Conclusion

The results of the investigation confirm the clinical impression that idiopathic renal acidosis of infancy is a self-limiting disease if the affected children are brought safely through the acute phase. In particular it shows complete recovery of the ability to secrete an acid urine (Fig. 2) and to form ammonia (the latter ability being present but impaired during the acute illness) (Latner and Burnard, 1950). From these results it appears that renal acidosis associated with nephrocalcinosis in older children and adults is a different condition from infantile renal acidosis and that the one does not lead to the other.

This work was done while one of us (E.U.B.) was holding the Ashby Memorial Research Scholarship of the University of Manchester.

We should like to thank Dr. O. Wrong for permission to include his figures for adults in the graph and for his co-operation, and Miss V. K. Wilson for advice and assistance on biochemistry.

REFERENCES

Butler, A. M., Wilson, J. L. and Farber, S. (1936). *J. Pediat.*, **8**, 489.
Carré, I. J., Wood, B. S. B. and Smallwood, W. C. (1954). *Arch. Dis. Childh.*, **29**, 326.
Davies, H. E. F. and Wrong, O. (1957). *Lancet*, **2**, 625.
Doxiadis, S. A. (1952). *Arch. Dis. Childh.*, **27**, 409.
Hartmann, A. F. (1939). *Ann. intern. Med.*, **13**, 940.
Kelsey, W. M., Reinhart, J. B. and Fishel, J. (1950). *Pediatrics*, **5**, 689.
Latner, A. L. and Burnard, E. D. (1950). *Quart. J. Med.*, **19**, 285.
Lightwood, R. (1935). *Arch. Dis. Childh.*, **10**, 205.
——, Payne, W. W. and Black, J. A. (1953). *Pediatrics*, **12**, 628.
Scott, J. A. (1955). *Report on the Heights and Weights of School Pupils in the County of London in* 1954. London County Council.
Tanner, J. M. (1958). In *Modern Trends in Paediatrics*, (Second Series) ed. A. Holzel and J. P. M. Tizard. p. 325, Butterworth, London.
Wrong, O. and Davies, H. E. F. (1959). *Quart. J. Med.* In the press.

APPENDIX

Case History

R.M., a first-born male child, was born on September 15, 1947, and was admitted to the Royal Manchester Children's Hospital at the age of 7 months on account of a two-month history of anorexia, loss of weight, fretfulness, constipation and intermittent vomiting. The pregnancy and delivery had been uneventful, and birth weight was 8¾ lb. He had been breast fed for only 2 weeks, and was then changed to National Dried Milk on which he continued to thrive until he was 5 months of age. Thereafter he lost 1 lb. 10 oz. in weight in five weeks. When seen at 7 months he weighed only 14 lb. 6 oz. Apart from obvious loss of weight and dehydration, the only abnormal feature was a flush over the malar bones unassociated with pyrexia. Following admission he developed an irregular pyrexia and his weight remained stationary. Investigations included a Mantoux test, 1 in 1,000, which was negative, radiographs of chest and wrist which were both normal, haemoglobin 78% (11·5 g.%) with colour index 0·93, white cell count 12,000 with normal differen-

tia[illegible] Throughout his stay in hospital there was constant slight albuminuria and the urine specific gravity never rose above 1,010. He had intermittent pyrexia, and urine culture twice yielded *Strep. faecalis* which cleared following courses of sulphadimidine and penicillin. The urine was usually neutral or alkaline but on one occasion was acid to litmus. Blood urea was 57 mg. % on admission and rose to 105 mg. %. Intravenous pyelogram showed poor excretion of dye but no obvious renal abnormality. In the last week in hospital his weight gain improved and when discharged on June 17, 1948, he weighed 15 lb. 4 oz. From July 6 to July 28, 1948, he was an in-patient at Birmingham Children's Hospital. There he was found to have hyperchloraemia (698 mg. % as sodium chloride), a low alkali reserve (25·5 vol. %) and a serum calcium of 11·2 mg. % and a diagnosis of infantile renal acidosis was made. The urine still contained a trace of albumin and some pus cells, and the ammonia content of a 24-hour specimen was only 0·11 g. By this time there was a marked improvement in his clinical condition and his blood urea had fallen to 50 mg. %. Treatment with alkalis was therefore not considered necessary. He took his feeds well and gained weight steadily and when discharged at the age of 10½ months he weighed 17 lb.

R.M. was seen as an out-patient in Manchester at regular intervals during the next 5½ years. His progress was rather slow until he reached 2 years of age but thereafter his height and weight were well up to the average for his age and no abnormality was found on physical examination, which included blood pressure estimation. His blood urea, however, remained above normal except on one occasion in 1954 when it was 29 mg. %. When reviewed in 1957 at the age of 9 years, 10 months, his height and weight were close to the 90 percentile for his age and he appeared to be very well. Blood pressure was 95/60 and a radiograph of the abdomen revealed no renal calcification. Urine examination, including microscopy, showed no abnormality and the concentration and dilution test gave a specific gravity range of 1,022 to 1,002 after 13 hours' deprivation of fluid. In the acidification test his performance was well up to the average, the urine pH falling to 5·2 after three hours and the rate of ammonium excretion being well within the normal range for that weight (see Fig. 1). Serum electrolytes were within normal limits but blood urea was still 43 mg. %; six months later this had fallen to 38 mg. % while the urea clearance was 72% of average normal function. In this boy, therefore, renal tubular function now appears to be restored to normal but there is still some doubt about glomerular function. The height to which the blood urea rose in the acute phase of the illness suggests that there may have been some glomerular damage at that time, and that this illness may not have been a pure idiopathic renal acidosis of infancy.

DIASTEMATOMYELIA

BY

C. C. MICHAEL JAMES and L. P. LASSMAN

From the Departments of Orthopaedic Surgery and Neurological Surgery, Newcastle upon Tyne General Hospital

(RECEIVED FOR PUBLICATION MAY 12, 1958)

Diastematomyelia is a rare condition and in our opinion is tending to be recognized more frequently by the radiologist, but its clinical significance does not appear to have been stressed. The purpose of this communication is to describe a case of this syndrome and to emphasize the importance of surgical treatment.

The term diastematomyelia is used to designate a congenital condition in which the spinal cord is divided longitudinally into two portions over some part of its extent. According to Cameron (1957) the term diastematomyelia was originally used by Hertwig in 1892 to describe a split in the spinal cord which occurred as a result of experimental interference with frogs' ova. Diastematomyelia becomes of clinical importance when there is a septum across the neural canal passing antero-posteriorly between the two portions of the spinal cord. The septum may be of bone, fibrous tissue, fibro-cartilage, or a mixture of these; it transfixes the spinal cord and divides partially or completely the neural canal antero-posteriorly in the midline. It most commonly affects the lower thoracic and lumbar regions but it has been described in the thoracic region as high as T.V.2 (Cowie, 1951). A similar septum may be found, in the absence of diastematomyelia between the nerves of the cauda equina, some of which may be adherent to the septum and diverted from their normal course. It has been found to be associated with other congenital anomalies of the vertebral column, particularly spina bifida, often of the spinal cord as in myelocele and occasionally with dermoid cysts (Matson, Woods, Campbell and Ingraham, 1950).

Some authors prefer the term diplomyelia for this condition because they regard it as the result of an attempt at twinning (Herren and Edwards, 1940; Benstead, 1953) but Cameron (1957) suggests that it is a failure of development associated with the delayed closure of the human equivalent of the blastopore. Cameron also, with Kapsenberg and Lookeren (1949), regards the presence of a septum as being due to the inductive influence of the split spinal cord upon the closely associated mutual development of dura and bone column, the septum being an abortive attempt at the formation of a midline neural arch by inversion of the laminae.

A septum is liable to fix the spinal cord so that traction is exerted on it as the vertebral column grows in length relatively faster than the cord, a process usually described as 'ascent' or 'migration' of the cord. The effect of transfixion becomes more marked with growth and, if it is severe and unrelieved, paralysis is likely to ensue owing to tension upon the cord itself, upon the nerve roots, and possibly owing to reduction of blood supply by traction on the blood vessels. A septum, therefore, is likely to be the cause of a progressive neurological deficit in the lower limbs, disparity of size of the lower limbs, incontinence, paresis, trophic changes and even paralysis.

The condition is usually recognized in childhood but has also been reported in adults in their twenties. The presenting symptoms are usually progressive weakening or failure to develop strength in a lower limb, or deterioration of gait. Occasionally the parents have brought the child because they were anxious about the presence of a hairy patch or naevus on the back and a radiograph has demonstrated diastematomyelia (Matson *et al.*, 1950).

Case Report

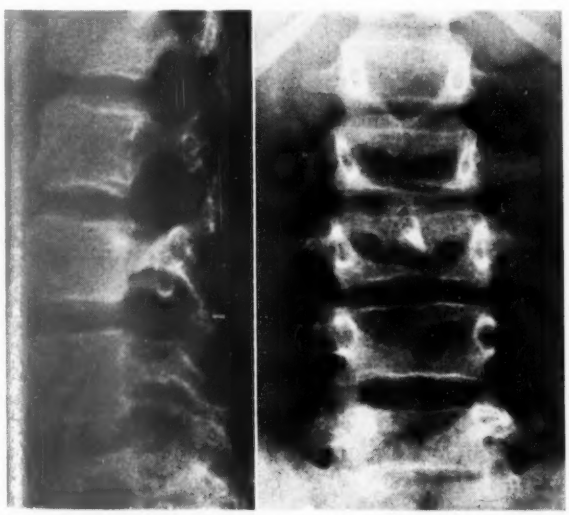
A girl, aged 10 years, attended an orthopaedic clinic complaining of discomfort in the right fourth toe in relation to the plantar aspect of the metatarso-phalangeal joint. No obvious cause for this was found but the appearance of the lower limbs attracted attention. The legs were of equal size and the feet normal except that the right foot was slightly smaller than the left. The skin of the legs and feet was livid and the circulation poor although it was a warm day. The whole appearance resembled that commonly seen in children with a myelomeningocele. Her mother said the girl frequently suffered from chilblains and that she always had 'very poor circulation in her legs', which went blue and mottled very easily; they never went 'dead' or painful. There was no history of bladder disturbance.

On examination of the back there was no abnormality evident except an excess of sparse hair over the lumbosacral area. The right plantar reflex was equivocal on that day but on the next examination four weeks later was found to be definitely flexor. There was no weakness in the lower limbs. She was small for her age; her three sibs, all younger, were of normal height. On admission to hospital two months later her condition on examination was unchanged but it was not possible to elicit her right plantar reflex: there was no demonstrable loss of sensation. Diagnosis was confirmed by radiological appearance and by myelography.

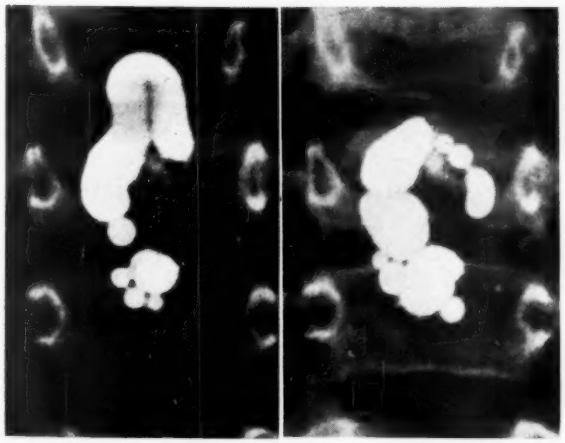
Plain radiographs showed spina bifida of L.V. 3, 4 and 5, and S.1. There was also a dense bony opacity situated in the midline and apparently extending from the spinous process of L.V.2 to the posterior aspect of the body of L.V.3 (Figs. 1 and 2), which was confirmed by tomography as being a midline bone spur. On myelography, the myodil column divided about 1 cm. below the bony spur and united again above the spur. A narrow, negative midline shadow was present for about 1·5 cm. above the spur (Figs. 3 and 4).

Operation. The spinous processes were removed, starting two segments lower than the expected site of the lesion. On removal of the spine of L.V.2 a bone pillar was displayed transfixing the cord. The pillar was extradural and closely invested by the dura; it was firmly attached posteriorly at the peak of the neural arch. The pillar at its narrowest was oval in section, 4 mm. in length (cranial-caudal) and 3 mm. wide. Its anterior attachment to the body of L.V.3 was not firm and was presumably fibrous.

The dura was opened, showing a division of the spinal cord into two equal parts, the split extending cranially from the pillar for 1·5 cm. All the roots were lateral to the cord. The split did not extend caudally from the pillar. There was a thin fibrous band ascending to a higher level within the dura and above the bony pillar; the band was divided and the bone removed, piecemeal and with difficulty, through the very small hiatus in the cord since it was not possible to rotate the cord without dividing the nerve roots.



FIGS. 1 and 2.—Survey views of the lumbar spine showing the bone spur lying in the sagittal plane.



FIGS. 3 and 4.—Myelography demonstrates the separation of the myodil above the spur by the fibrous membrane (Fig. 3) and below the spur by its base. (The spur is ringed in Fig. 3.)

The cord was free at the end of operation; it had not ascended when released. The conus medullaris was situated 1½ cm. below the pillar. Closure was normal.

The post-operative course was uneventful; for a few hours the patient had pain in the left buttock. About three months later she complained of pain in her left great toe, but this rapidly subsided.

Five months after operation, her legs were of normal appearance as far as her circulation was concerned; she had no pain but clinically there was slight weakness of the left flexor hallucis longus.

Nine months after operation, there was no abnormality to be found. The circulation was improved, but was still not completely normal, and she had had no chilblains.

Discussion

The preliminary radiographical examinations had quite clearly demonstrated what was actually found at operation. The curious 'narrow, negative midline shadow' seen in the myelogram was found to be due to the thin fibrous band within the dura lying in the midline cleft in the spinal cord. It was attached to the dura internally, both at the cranial edge of the bone pillar and posteriorly. It was not possible at operation to determine clearly if the fibrous band was also attached to the dura overlying the posterior aspect of the vertebral body. The myodil column divided about 1 cm. below the bony spur because of the obliquity of the spur whose anterior attachment was more caudal than its posterior attachment.

As Matson *et al.* (1950) have pointed out, the septum passing antero-posteriorly across the spinal canal may not be sufficiently calcified in young children to show on a radiograph. If the presence of a septum is suspected, repeat examinations will be necessary later on, or, as our findings showed, the condition can be demonstrated by myelography and unnecessary delay in making a diagnosis thereby avoided. Also there are two other radiographical pointers which can indicate the possibility of diastematomyelia: increased inter-pedicular distance and spina bifida. Herren and Edwards (1940) found that the spinal canal is widened where there is congenital abnormality of the cord, and both Neuhauser, Wittenborg and Dehlinger (1950) and Cowie (1951) have pointed out that increased inter-pedicular distance without pedicular erosion is a sign of congenital spinal cord abnormality and is present in cases of diastematomyelia. If the views of Kapsenberg and Lookeren (1949) and Cameron (1957) on developmental anomalies of the spinal cord are accepted, it seems to be impossible for diastematomyelia, with or without a septum, to occur without a very close relationship to spina bifida.

The decision to operate was based on the knowledge that the effects of traction on the cord were likely to be progressive. There was no reason to delay by watching to find out if indeed there would be progressive deterioration of circulation and muscle tone in the lower limbs, particularly since these effects may not appear until the age of 23 years; they may be rapid and irreversible. It is these facts which make it important to make the diagnosis early and to act to correct the anomaly. In this patient, the conus medullaris of the spinal cord was lying at a lower level (lower border of L.V.4) than is normally found at the time of birth, a state which was not demonstrated pre-operatively, and its retention at this level had so far produced remarkably little neurological result.

It is not possible to explain satisfactorily the mildness of the neurological effects of the lesion. They were not in themselves causing any disability but were noticed quite by chance during a clinical examination for another complaint. The circulatory deficiency had been present for many years. There are, however, various factors to be considered, the age at which there is the greatest differential rate of growth of bone column and spinal cord, the point of fixation of the cord and the firmness with which the cord is tethered.

There does not appear to be any information about the levels of the conus in the years between birth and completion of growth, and it is not known whether the bone column and spinal cord maintain the same rate of growth relative to each other throughout the growing period or whether, for example, they grow equally for a period and then the bone column grows much faster. Since the bone column and spinal cord are developed in close association with each other in embryo it is likely that they are closely associated throughout the post-natal growing period and it is reasonable to argue from what is known about the growth of the skeleton. The period of greatest increase in height is around puberty and it is likely to be at this time that the 'ascent' of the cord is most rapid. The dura and arachnoid normally grow at almost the same rate as the bone but keep a definite relationship with the spinal cord in that the nerve roots in the adult have only a short course within the main dural sheath before acquiring their own individual sheaths. The dura therefore is itself not normally under any great traction force and actively lengthens as the bone column grows, just as do the spinal nerves (Streeter, 1919). The spinal cord also grows, but more slowly than the bone; its length at the end of the growth period is greater than at birth. If for any reason the bone age of the individual is less than normal, the ascent of the cord will be equally delayed. In the present case the bone age was normal and the child had not attained puberty. Since there is relatively less ascent of the spinal cord at the cranial end than at the caudal end of the vertebral canal, as shown by the obliquity of the nerve roots in their course to the neural foramina, fixation caudally is more likely to produce neurological signs. In the present case the point of fixation of the cord was relatively low in the bone column and relatively high in the spinal cord but the cord was not immobilized. The bone septum was situated in a division of the spinal cord of about 1·5 cm. length and was at the caudal end of the division; when the septum was removed, the cord did not alter its position. This suggests that the spinal cord had already been able to ascend to a small extent but would in a short time have been prevented from ascending further except at the expense of the elasticity of the cord tissues with resultant pressure effects and increased neurological signs.

As already mentioned, this child was small for her age. Using the crown-rump measurement, but not as accurately as Provis and Ellis (1955), our case was 64·9 cm. long in the stem which corresponds with the average of 64·5 cm. and 66·9 cm. found in Edinburgh girls at mean ages of 6·54 and 7·49 years respectively. Her bone age as estimated by radiographs of her elbows and wrists was the same as her true age so that the delay in development of

neurological signs cannot be ascribed to late bone growth.

Summary

Diastematomyelia is a congenital division of the spinal cord in its length. A case is described where there was an associated bone spur passing across the spinal canal in the lumbar region between the two halves of the cord.

The operation findings are described and discussed in relation to the clinical signs and to the radiological appearances. Preliminary tomography and myelography clearly demonstrated the anatomical state but the clinical signs bore no relation to the abnormal position of the spinal cord, fixed as it was in the vertebral canal by a bone septum, with the conus medullaris at a level below that normally found at birth. The various factors associated with this abnormality are discussed in detail in an attempt to explain the paucity of neurological signs in this case.

The need for early diagnosis and preventive surgery in cases of diastematomyelia transfixed by a septum is stressed.

Our grateful thanks are due to Dr. Sheila Cameron, radiologist, and Dr. Gordon Gryspeerdt, neuroradiologist, Newcastle General Hospital, for their assistance in the diagnosis of this case.

References

Benstead, J. G. (1953). *J. Path. Bact.*, **66**, 553.
Cameron, A. H. (1957). *Ibid.*, **73**, 195 and 213.
Cowie, T. N. (1951). *Brit. J. Radiol.*, **24**, 156.
Herren, R. Y. and Edwards, J. E. (1940). *Arch. Path.* (*Chicago*), **30**, 1203
Kapsenberg, J. G. and Lookeren, J. A. van (1949). *Acta Anat.* (*Basel*), **7**, 366.
Matson, D. D., Woods, R. P., Campbell, J. B. and Ingraham, F. D. (1950). *Pediatrics*, **6**, 98.
Neuhauser, E. B. D., Wittenborg, M. H. and Dehlinger, K. (1950). *Radiology*, **54**, 659.
Provis, H. S. and Ellis, R. W. B. (1955). *Arch. Dis. Childh.*, **30**, 328.
Streeter, G. L. (1919). *Amer. J Anat.*, **25**, 1.

A CASE OF CUSHING'S SYNDROME IN AN INFANT

BY

E. GOLDBLATT and A. H. SNAITH

From The Hospital for Sick Children, Great Ormond Street, London

(RECEIVED FOR PUBLICATION JUNE 2, 1958)

Cushing's syndrome is rare in infancy. The present case is reported because of the unusual pathology and because to our knowledge this is the first case in which bilateral adrenalectomy has been carried out in an infant.

Case History

The patient, a girl, was born after a normal full-term pregnancy. Her birth weight was 8 lb. 4 oz. (75th percentile) and at 12 months, when she was apparently normal, her weight was 24 lb (75th percentile). Two months later she was brought to hospital because of her rapid gain in weight and increasing irritability. During this period she had gained 4 lb. 14 oz. and weighed 28 lb. 14 oz. (97th percentile). Thus the onset of her condition was short and dramatic (Figs. 1 and 2).

On examination (Fig. 2) she was obese, with a buffalo hump, her face was plethoric and she had an acneiform rash. There was slight hirsutes of the face and back. No striae were present. She had a small umbilical hernia. The height was 30 in. and the span 30¼ in. (50th percentile). The blood pressure was 140/90 mm. Hg (normal average 96/66 mm. Hg). On radiographic examination the bone age was normal and there was no osteoporosis of the spine, skull or long bones. An intravenous pyelogram suggested that there might be enlargement of the right adrenal. Glucose tolerance test: fasting blood sugar, 89 mg. %; sugar readings were 111, 111, 117, 137 and 125 mg. %. The serum sodium was 147 mEq./1, the serum potassium 4·4 mEq./1, the blood cholesterol 154 mg. %; serum proteins were 7·0 g. % and the α and γ globulins were slightly raised. The haemoglobin was 110% (16·3 g. %), and the white count normal except that no eosinophils were present. R.B.C. were 4,720,000/c.mm. Details of steroid excretion are given in Table 1.

In the expectation that an adrenal tumour would be found an exploratory laparotomy was undertaken by Mr. D. Innes Williams. Cortisone 25 mg. per day was given orally in divided doses for two days prior to the operation. Both adrenal glands were found to be slightly enlarged and nodular. The left adrenal was

FIG. 1.—Apparently normal at 8 months old.

FIG. 2.—On admission aged 14 months.

TABLE 1

STEROID EXCRETION IN GIRL AGED 14 MONTHS WITH CUSHING'S SYNDROME DUE TO BILATERAL MULTIPLE ADRENOCORTICAL ADENOMATA

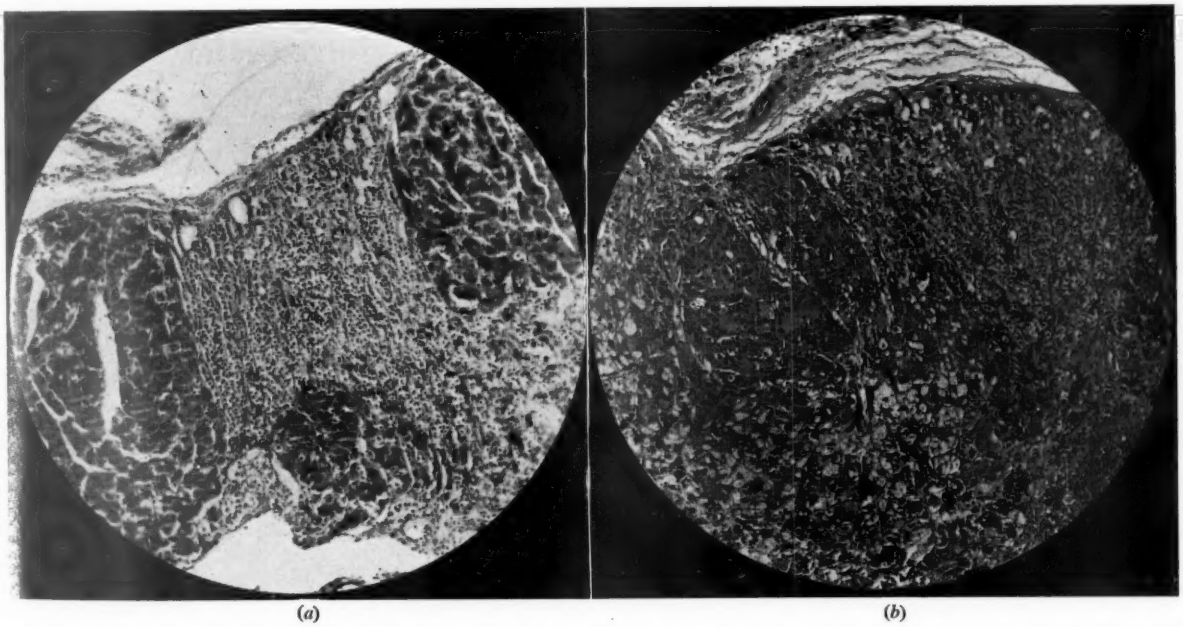
Stage and Treatment	17-Keto-steroids (mg./24 h.)	17-Hydroxy-steroids (mg./24 hr.)	17-Ketogenic-steroids (mg./24 hr.)
Normal for age ..	0·5	1·2	2·0
Before first operation ..	2·9	9·7	21·3
Following removal of left adrenal ..	2·4	4·2	6·0
After 5 days of:			
ACTH 25 mg./day ..	2·9	9·9	15·8
9-*a*-fluorohydrocortisone 500 µg. b.d. ..	1·6	10·4	7·5
Methyl testosterone 10 mg./day ..	3·4	3·4	8·8
Stilboestrol 1 mg. per day	2·4	3·8	6·0

Before operation aldosterone excretion was < 2 µg./24 hours, total oestrogen excretion $< 1{\cdot}0$ µg./24 hours and urinary gonadotrophins < 5 m.µ./24 hours (within normal limits).

removed (Figs. 3, 4a and 4b). The liver was large and fatty. There was a moderate hypokalaemic alkalosis for the first week after operation, during which time 1 g. of potassium chloride per day was given and cortisone was continued in the same dosage as before. Otherwise, the post-operative course was uneventful. After further investigation it was decided to remove the right adrenal. The day before the second operation 50 mg. of cortisone were given by mouth and on the day of the operation 50 mg. b.d. of cortisone were given intramuscularly. The post-operative course was uneventful, cortisone was gradually reduced and the patient was discharged two months later, by which time her appearance had become much more normal. At present she is being maintained on 20 mg. of cortisone acetate and 1 g. of salt a day. Three months after discharge from hospital she was again admitted, suffering from avulsion of two finger tips which she had caught in a train door. She withstood this trauma without any evidence of metabolic upset.

FIG. 3.—Left adrenal. Note multiple nodules.

Further Investigations. After removal of the left adrenal there was a decline in steroid excretion but the clinical condition remained unchanged. The influence of ACTH, fluorohydrocortisone, methyl testosterone and of stilboestrol on steroid excretion was then studied (Table 1) and it was concluded that none of these hormones, in the dose given, strikingly influenced secretion by the remaining gland. The great increase in



(*a*) (*b*)

GS. 4a and 4b.—Photomicrographs of adrenal glands × 60, illustrating multiple adenomatous nodules and intervening areas of atrophic cortical tissue.

17-hydroxysteroid excretion which might have been expected in a case of adrenal hyperplasia did not occur after the administration of A.C.T.H.

Discussion

In children Cushing's syndrome is usually due to tumour (Guin and Gilbert, 1956); a case of adrenal hyperplasia has recently been described in a child aged 8½ by Hubble and Illingworth (1957) and in a case of the same age reported by Chute, Robinson and Donohue (1949) multiple bilateral adenomatous nodules of the type present in this case were found at autopsy. The aetiology of adrenal hyperplasia with Cushing's syndrome remains obscure. Elevated blood ACTH levels have not been demonstrated and it is possible that the adrenal glands are unduly sensitive to ACTH. Jailer, Longson and Christy (1956) have suggested that the pituitary secretes in excess a hypothetical corticotrophin-potentiating substance. The response to ACTH administration in the present case was moderate, and the histological appearance of normal adrenocortical cells adjacent to nodules of abnormal cells could be compatible with the view that secretion by the abnormal cells was in fact to a large extent autonomous.

In view of the age of the patient the possibility that the hyperplastic glands were the result of abnormal development of the foetal cortices was considered. It has been suggested that the human foetal cortex may be dependent upon pituitary gonadotrophins, as is the 'X' zone in the mouse (Chester Jones, 1957). No change in steroid excretion was observed to follow the administration of stilboestrol or methyl testosterone, which might have been expected to suppress gonadotrophin secretion. No gonadotrophins could be detected in the urine.

Irradiation of the pituitary gland was not considered appropriate treatment in view of the age of the patient, since to be effective such treatment might interfere with the secretion of other trophic hormones; moreover the response to ACTH did not indicate that a diminution of ACTH secretion would necessarily be of great benefit.

Adrenal enucleation is a procedure which may be successfully carried out in the rat, a new cortex regenerating from solitary cortical cells which adhere to the capsule (Chester Jones, 1957). Such a procedure, if practicable, would have been of interest in this patient since it might have been observed whether normal or abnormal cells regenerated, and thus, perhaps, some light might have been thrown on the fundamental aetiological problem in adrenal hyperplasia. However, it does not seem surgically possible to remove the cortex so as to leave the capsule behind as an intact bag, as in the rat. Furthermore, there is some doubt whether adrenal regeneration occurs after enucleation in the human subject (Dempster, 1955). Consequently, total adrenalectomy, which is the treatment of choice in the older patient was undertaken here with the intention of maintaining the patient on cortisone for the rest of her life. The long-term prognosis remains to be seen (Fig. 5).

FIG. 5.—Five months after total bilateral adrenalectomy.

We wish to thank Dr. A. P. Norman for permission to publish this case, Dr. I. Chester Jones with whom we discussed the possibility of adrenal enucleation for his advice, and Mr. Derek Martin, of the Department of Medical Illustration, for the photographs.

REFERENCES

Chute, A. L., Robinson, G. C. and Donohue, W. L. (1949). *J. Pediat.*, **34**, 20.
Dempster, W. J. (1955). *Brit. J. Surg.*, **42**, 540.
Guin, G. H and Gilbert, E. F. (1956). *A.M.A. J. Dis. Child.*, **92**, 297.
Hubble, D. V. and Illingworth, R. S. (1957). *Arch. Dis. Childh.*, **32**, 285.
Jailer, J. W., Longson, D. and Christy, N. R. (1956). *J. Clin. Endocr.*, **16**, 1276.
Jones, I. Chester (1957). *The Adrenal Cortex*, p. 112 Cambridge.

EPIDEMICS OF GASTRO-ENTERITIS ASSOCIATED WITH *ESCH. COLI* O119 INFECTION

BY

K. HUGH-JONES* and G. I. M. ROSS†
From the Westminster Children's Hospital and Westminster Medical School

(RECEIVED FOR PUBLICATION JUNE 23, 1958)

Introduction

Three outbreaks of gastro-enteritis associated with the presence of *Esch. coli* O119 in the stools occurred among the infants in a baby ward between September, 1954, and February, 1956. In these epidemics altogether 45 infants were found to be infected. During the whole period the organism was also isolated from 13 out-patients and three children in other wards. This paper describes the frequency with which the organism was found in the stools of those at risk, the association between this finding and symptoms, some biochemical and antigenic characteristics of the *Esch. coli* O119, together with the results of antibiotic and sulphonamide sensitivity tests and a note of comparison with the clinical response.

Bacteriological Methods

Swabs of faeces were taken from each infant on admission and thereafter on every Monday and Thursday while in the ward.

Cultures. The routine swabs were plated on MacConkey agar and desoxycholate citrate agar. Swabs from those known to have diarrhoea were inoculated also into selenite F broth from which subcultures were made on to desoxycholate citrate agar after 24 hours' incubation. All the *Esch. coli* O119 strains isolated were stored on Dorset egg slopes for further tests.

Antigenic Identification. (*a*) O ANTIGENS. During the first year of this investigation all faecal specimens were examined for the presence of 10 different O types of *Esch. coli*. These types were O25, O26, O55, O86, O111, O114, O119, O125, O126 and O128. The antisera initially used for this purpose were most kindly supplied by Dr. J. Smith of Aberdeen. Later, sera prepared in this laboratory and polyvalent and individual O antisera supplied by the Standards Laboratory of the Central Public Health Laboratory were also used.

Mixtures of lactose-fermenting colonies of gram negative bacilli on MacConkey agar were tested for agglutination on Murray slides with these sera. Pure colonies of the various types of *Esch. coli* were then selected by slide agglutination, if necessary after further subculture. Their identity was finally determined by tube agglutination tests showing that, after boiling for one hour to destroy K antigen, they were agglutinated to the titre of the O antiserum. One of the earliest strains of O119 isolated in the first epidemic was kindly confirmed as such by Dr. Joan Taylor.

After the first year of this study tests were made routinely only for *Esch. coli* types O26, O55, O111, O119 and O128 as the numbers of the other types encountered did not justify the considerable extra work involved in searching for them. In all cases of gastro-enteritis, however, when tests were negative for these five types of *Esch. coli* and for salmonellae and shigellae, the additional antisera against the other five *Esch. coli* types were used to try and identify a possible pathogen in the stools.

(*b*) H ANTIGENS. One strain of O119 isolated in the first epidemic was found by Dr. Joan Taylor to be type H6. Then, using antiserum kindly provided by Dr. Taylor, other strains were typed by tube agglutination. Motility was readily obtained with only one or two passages through Craigie tubes.

Biochemical Tests. The fermentative ability of all the strains of typable *Esch. coli* isolated during the period of the study was tested in 1% peptone water sugars. Sugars were incubated up to 21 days if acid had not been produced before that time. Tests such as indole, Voges-Proskauer and methyl red were carried out by the standard procedures described in Topley and Wilson (1946).

Sensitivity Tests. Antibiotic sensitivity tests were performed immediately after the isolation of each strain of typable *Esch. coli* using tablets (Evans Medical Supplies Limited) containing penicillin, streptomycin, aureomycin, terramycin and chloramphenicol. Subsequently the exact bacteriostatic concentrations of these antibiotics, and of neomycin, polymyxin, tetracycline and sulphathiazole were determined by plating each organism on agar containing doubling concentrations of these agents. The sulphonamide sensitivity tests were made

* St. Bartholomew's Hospital, London, E.C.1.
† Ashford Hospital, Ashford, Middlesex.

using the medium recommended by Jewell and Pearmain (1954) but with 2% horse blood added.

General Background

These epidemics occurred in the baby ward of Westminster Children's Hospital. The ward consisted of 11 separate cubicles, one of which sometimes took two infants and seven of which were nursing-mother flats. All these cubicles were entirely separate rooms, each with a wash basin. Individual nurse's and doctor's gowns were provided for each baby and the usual barrier nursing techniques were used whenever attending to the children. Every effort was made to avoid admitting cases of gastro-enteritis to the ward, but during the period under review four infants were finally diagnosed as such and a pathogenic *Esch. coli* other than O119 demonstrated in their faeces. Two were infected with an O55, one with an O111 and one with an O128.

Clinical Findings During the Survey

First Epidemic. PRELIMINARY SURVEY. During July and August, 1954, several babies in the ward developed gastro-enteritis and in September testing for pathogenic *Esch. coli* was first begun. Four children (Cases 1-4), who had or had had gastro-enteritis recently, were found to be excreting *Esch. coli* O119. Because of this the ward was shut and this investigation begun.

MAIN SURVEY. The ward was closed for four days in Week 2 (Fig. 1) when each cubicle was scrubbed, fumigated with formaldehyde and then aired for 24 hours. On opening the ward entirely new patients were admitted. From this point onwards the routine swabbing on admission, and thereafter twice weekly, was begun and daily enquiry made regarding each infant's stools. In all, some 370 swabs were examined from 51 infants. During the first week that the ward was open (Week 3) all the children had negative swab results.

Within 12 days of reopening the ward (Week 4) *Esch. coli* O119 was demonstrated in the stools of a breast fed baby (Case 5) and the steady spread of the infection can be followed from case to case in Fig. 1. A further 22 babies were infected. Case 3 was readmitted in the ninth and reinfected in the eleventh week (Fig. 1) bringing the total to the

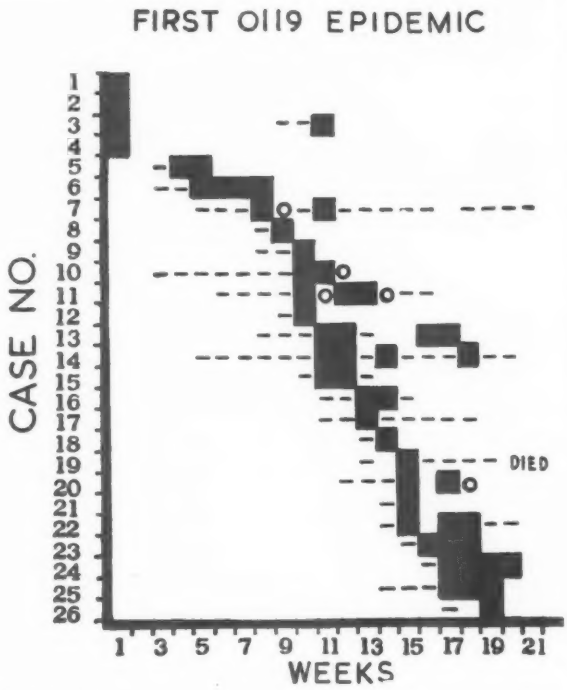


FIG. 1.—The results of culture of the faeces in each case in each week of the epidemic: ■ *Esch. coli* O119; — No typable *Esch. coli*; O No *Esch. coli*.

23 cases shown in Table 1. Fig. 2 shows the number of babies in the ward during each week, the number with positive cultures and the number with definite gastro-enteritis.

Once the organism had been demonstrated again after reopening the ward it will be seen that at least

TABLE 1

Group	Criteria			Number of Infants			
	Admission Swab	Further Swabs	Clinical Evidence of Gastro-enteritis	First Epidemic	Second Epidemic	Third Epidemic	Total
I	Negative	Negative	None	28	33	14	75
II	Negative	Positive	None	8	4	2	14
III	Negative	Positive	Mild	5	2	0	7
IV	Negative	Positive	Definite	10	8	2	20
Total infants at risk in each epidemic				51	47	18	116

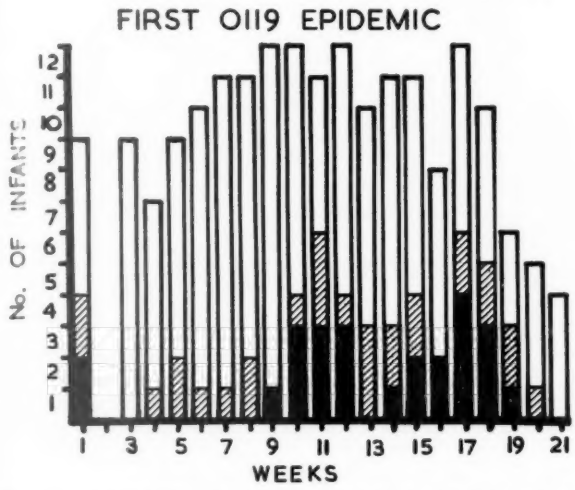


FIG. 2.—The total number of infants, those with *Esch. coli* O119 in faeces and those with gastro-enteritis, in each week of the epidemic: ■ Number with definite gastro-enteritis; ▨ Number with *Esch. coli* O119 in faeces; □ Total number of infants in ward.

one child was excreting it in each of the remaining weeks of the epidemic. There were two peaks (Weeks 11 and 17) during which 50% of the children had positive cultures. Because of the second peak of cross-infection no further babies were admitted to the ward, and so the epidemic died out at the end of January, 1955, after more than 20 weeks and the ward was then closed. During this epidemic one child attending Out-patients with gastro-enteritis was found to be infected with *Esch. coli* O119.

Interim Period between First and Second Epidemics. The ward was shut for two weeks for thorough cleaning and repainting; during this time there was a vigorous overhauling of nursing procedures and structural improvements were planned. Although

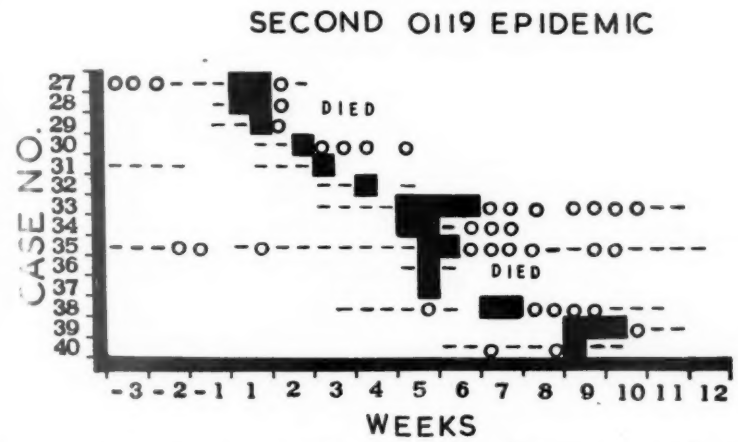


FIG. 3.—The results of culture of the faeces in each case in each week of the epidemic: ■ *Esch. coli* O119; — No typable *Esch. coli*; O No *Esch. coli*.

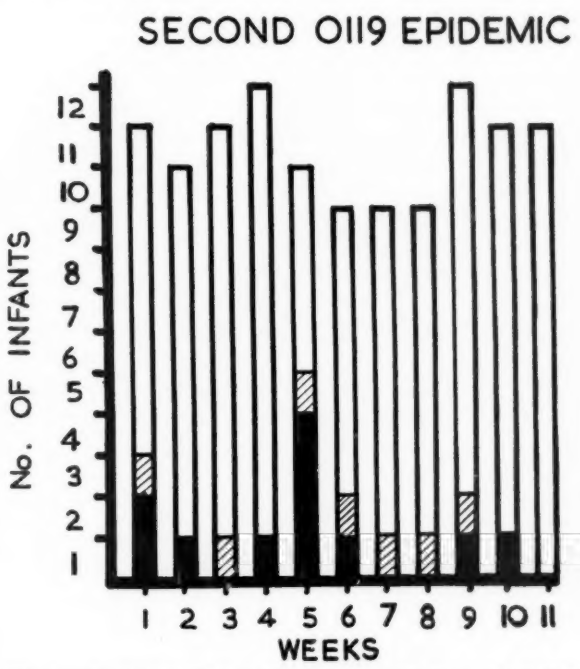


FIG. 4.—The total number of infants, those with *Esch. coli* O119 in faeces and those with gastro-enteritis, in each week of the epidemic: ■ Number with definite gastro-enteritis; ▨ Number with *Esch. coli* O119 in faeces; □ Total number of infants in ward.

no serious deficiencies in the methods of preventing cross-infections were brought to light, the discussion and general awareness no doubt improved individual techniques. It was also decided for the future that even the mildest case of gastro-enteritis due to pathogenic *Esch. coli* in that ward should be treated energetically with antibiotics.

The ward was reopened in February, 1955, and no case of *Esch. coli* O119 infection was detected for 15 weeks. During this period some 300 faecal samples from 48 infants were examined.

Second Epidemic. On June 6, 1955, two babies (Cases 27 and 28) developed gastro-enteritis and were found to have *Esch. coli* O119 in their stools on the same day (Week 1, Fig. 3). Fig. 3 shows the spread of the in-infection from case to case during the 10 weeks that the epidemic lasted. The organism was demonstrated in the faeces of at least one infant during each week except Week 8. During this week Case 38 continued to have diarrhoea though no *Esch. coli* was grown from his faeces; terramycin was being given at that time. Fig. 4

shows the number of babies in the ward, the number with positive cultures for *Esch. coli* O119 and the number with definite gastro-enteritis each week. The peak incidence of infection was in Week 5 when 50% of the children had positive cultures. In all, 14 infants were found to be infected.

The source of this infection was not known. It was unlikely that the organism had remained alive in the ward during the 18 weeks since the previous epidemic. The most likely origin was Case 27 who was admitted with whooping cough and treated with terramycin for the first three weeks. Towards the end of this course of treatment the child had some diarrhoea but this then stopped. Two weeks later, however, it recurred and *Esch. coli* O119 was first demonstrated in the faeces. Alternatively the organism may have been carried by a member of the staff from the first epidemic; or it may have been introduced into the ward, directly by the admission of an unidentified carrier, or indirectly by a member of the staff from a child with diarrhoea attending Out-patients. However, as no *Esch. coli* O119 was demonstrated either in the ward or from Out-patients before June 6 the source of infection must remain uncertain. During the epidemic the organism was found in the faeces of one infant in another ward and of five children, two in one family, attending Out-patients. It seems possible that the epidemic was part of a more widespread infection in the locality. The epidemic died out in the tenth week and thereafter till the ward was closed in Week 12 for structural alterations, *Esch. coli* O119 was not again demonstrated. During this epidemic some 180 faecal specimens from 47 infants in the ward were examined.

Interim Period between Second and Third Epidemics. This period was of 26 weeks during which over 800 faecal samples were examined. Eight infants were found to be infected with *Esch. coli* O119. One was admitted to a different ward from that in which the epidemics occurred and the others were out-patients. Seven had attended because of gastro-enteritis, the eighth was a 4-year-old symptom-free contact. Six of these cases occurred during November, 1955.

Third Epidemic. This epidemic began in February, 1956, and continued for 10 days. Four infants only in the baby ward were infected out of 18 at risk. One of these had been in the ward 10 days previously and presented with diarrhoea as an out-patient five days after the end of the epidemic. He was found to be excreting *Esch. coli* O119. It seems likely that he was infected while in hospital and only developed symptoms and positive stools after discharge. The infection appeared to have been introduced by an infant who was admitted with otitis externa. The first swab taken on admission showed the presence of the organism. This child was discharged home after five days in hospital. She was still excreting the organism in her stools but was always without any symptoms of infection. The other three infants had negative stools on admission and were presumably infected from the first child. Two of them had definite gastro-enteritis.

General Features

Incidence of Gastro-enteritis and Mortality in the Infected Infants. It can be seen from Table 1 that during the three epidemics in the ward 41 infants were found to be infected out of 116 at risk, and of those infected seven had mild symptoms and 20 definite gastro-enteritis. In this analysis Cases 1 to 4 are not included as they occurred during the preliminary survey.

In the first epidemic one child died of gastro-enteritis. In the second two died, both with severe congenital abnormalities. One had a myelomeningocoele and hydrocephalus and his death was accelerated by gastro-enteritis. The other had congenital heart disease and died aged 12 days in congestive heart failure.

Carrier State. The second group in Table 1 of 14 children who were excreting the organism but remained symptom-free supports the suggestion that a carrier state exists as it does in other intestinal infections. The finding of the two older symptom-free children aged 4 and 6 in Out-patients also supports this. Although these children are well in themselves they are a danger to others. This is especially so because the organism can often be demonstrated in almost pure culture from their stools. Such a child seemed to be the origin of the third epidemic.

Incubation Period. To try and estimate the incubation period the time relationship between the first positive culture and the development of symptoms was studied, and also the length of time at risk was calculated, i.e. in the ward before the first positive culture developed. Out of the 20 infants with definite gastro-enteritis, five developed symptoms on the day of the first positive culture, four one day after, two two days after and one three, four, nine, 10 and 11 days after. Four developed symptoms before their first positive culture, one on the day before and the others on the fifth, sixth and seventh days before.

Remembering that cultures were made only twice weekly, the fact that 13 out of the 20 developed symptoms within four days of the organism being demonstrated suggests a short incubation period. This is supported by the fact that 60% of those that did become infected were found to be excreting the organism within a fortnight, and 20% within five days, of exposure to infection in the ward.

Age. The age incidence in the epidemics can be seen in Fig. 5. Because this ward was for babies there were more children in the youngest age group; nearly 80% of the admissions were under 6 months. Most of those that became infected did so during their second to fifth months of life—a time when infants are particularly susceptible to infection, which may be related to the low level of gamma globulin in their blood. The oldest child whom we found to be infected with an O119 was aged 6 years; he was symptom free. The oldest with symptoms was aged 2½ years. Both were out-patients.

Feeding. About one-fifth of the 116 infants at risk were entirely breast fed while in hospital but of the 41 infected infants only one was breast fed and that child was symptom-free (Case 5). Although the mothers were nearly always admitted with their babies, the routine nursing procedures were still carried out by the staff.

Treatment. FIRST EPIDEMIC. Systematic treatment with sulphonamides or antibiotics of every infant found to be infected with O119 was not given, as the policy of treating all cases however mild, or all carriers, had not yet been accepted. Several infants received more than one course of treatment with one or more drugs while in hospital. These courses were given as treatment either for the original cause of admission or for gastro-enteritis.

Nine infants received sulphonamide orally, two streptomycin orally and seven penicillin systemically; all continued to excrete the organism while on treatment. One infant received chloramphenicol orally; he had negative swabs during treatment but was found to be excreting the organism again within seven days of stopping the drug.

SECOND EPIDEMIC. Four infants were discharged from hospital within a day or so of the organism being discovered in their stools and a fifth died; these were all untreated.

Seven infants with *Esch. coli* O119 infection were treated with terramycin and two with aureomycin followed by terramycin. The dosage of terramycin ranged from 10-16 mg./lb./day (mean 12 mg./lb./day) for four to 27 days (mean 12 days). In the nine treated patients all types of *Esch. coli* disappeared completely from the stools (Fig. 3). Once the stools

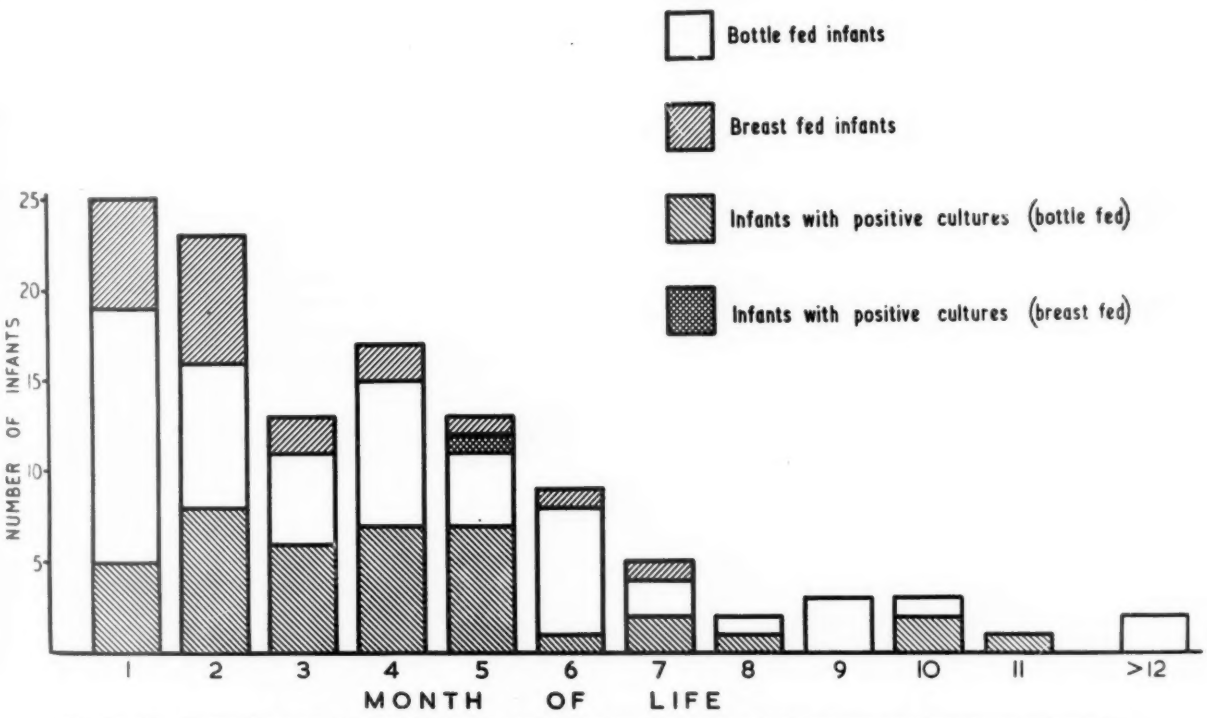


FIG. 5 —The age incidence of all the infants in the three epidemics, those with positive cultures and those breast or bottle fed.

had become negative for O119 they remained so during the time the infants were in hospital.

THIRD EPIDEMIC. The two children with symptoms were given terramycin, one receiving 50 mg./lb./day, the other 120 mg./lb./day. In both the cultures became negative. One had previously been treated with sulphonamide (125 mg./lb./day) for nine days with no effect.

Sensitivity of the *Esch. coli* O119 Strains to Antibiotics and Sulphonamides. The sensitivity of the *Esch. coli* O119 strains isolated from 53 of the 61 infected infants were tested. All 53 strains had similar sensitivities. The bacteriostatic concentrations of the different drugs are shown in Table 2.

TABLE 2
SENSITIVITY OF 53 STRAINS OF *ESCH. COLI* O119

Drug	Number of Strains Completely Inhibited by Lowest Concentration of Drug (μg. per ml. or units* per ml.)							(mg. per 100 ml.)	
	1	2	4	8	16	50	100	512	1024
Polymyxin* ..	22	31							
Terramycin ..		9	41	3					
Tetracycline ..		19	30	4					
Chloramphenicol			24	29					
Aureomycin ..			11	31	11				
Streptomycin ..			1	27	25				
Neomycin ..			12	41					
Penicillin* ..						28	25		
Sulphathiazole ..								11	42

All were sensitive to therapeutic concentrations of polymyxin, terramycin, aureomycin, tetracycline, chloramphenicol, streptomycin and neomycin and all were resistant to penicillin and sulphathiazole.

In view of the resistance to the sulphonamide it is not surprising that drugs of this group proved so ineffective during the first epidemic. Terramycin given during the second and third epidemics proved effective in eradicating the organisms from the stools. Yet in spite of this the second epidemic continued for 10 weeks.

Biochemical Characteristics of the *Esch. coli* O119 Strains Isolated. Strains isolated from 53 infants were tested. These were the same strains as those on which the sensitivity tests were made. All gave identical reactions. Glucose, maltose, mannitol, lactose, sucrose, arabinose and xylose were fermented with acid and gas production within usually three days. Dulcitol was fermented with acid and gas production after two to 10 days. Variable times in the fermentation of dulcitol were found with even the same strains in several different batches of dulcitol peptone waters. Sorbitol was fermented after one day. Adonitol, inositol, inulin and rhamnose were not fermented in 21 days. All strains were indole positive, urease negative, methyl red positive, Voges-Proskauer negative and failed to liquefy gelatin. Tests for mucus production and haemolysis were negative.

H Antigen Type of the *Esch. coli* O119. One of the earliest strains isolated in the first epidemic was found to be type H6 by Dr. Joan Taylor. We subsequently typed 57 strains isolated from different infants during the period of the investigation and found all to be type H6.

Bacteriological Findings in the Whole Period of the Investigation. The findings in 1,044 specimens from 400 children studied during the three epidemic periods are summarized in Table 3. During these

TABLE 3
FINDINGS IN 1044 FAECAL SPECIMENS FROM 400 INFANTS EXAMINED DURING THE THREE *ESCH. COLI* O119 EPIDEMICS

	Baby Ward		Other Wards	Out-patients
	O119+ve	O119−ve		
Infants (No.):				
studied	45	75	66	214
with *Esch. coli* O119 ..	45	0	2	6
with other pathogenic types of *Esch. coli* ..	5	6	8	15
Specimens (No.):				
total examined ..	412	186	116	330
with *Esch. coli* O119 ..	105	0	13	9
with no *Esch. coli* ..	80	30	14	61
with other pathogenic types of *Esch. coli* ..	15	11	9	18

periods a total of 127 specimens from 53 children were found to contain O119 while about 15% contained no *Esch. coli* of any type, usually because of antibiotic or sulphonamide therapy. In the intervals between the three epidemics another 1,224 specimens from 418 children were examined. Of these specimens 260 contained no *Esch. coli* and eight out of 72 specimens with pathogenic types of *Esch. coli* contained O119. In addition 782 faecal swabs from 76 newly born infants in three different maternity units, kindly provided by Dr. I. M. Anderson, were examined. These swabs were taken daily from birth till discharge from hospital. In only one of these swabs, taken from a baby without symptoms on the tenth day after birth, was a typable *Esch. coli* found and this was an O128.

Incidence of Other Types of *Esch. coli*. The incidence of other types of *Esch. coli* at Westminster Children's Hospital during the whole period of the

study is shown in Table 4. In all, 60 infants were found to be infected, of whom 18 were in the baby ward. During the first epidemic five of the 26 infants with O119 infection in the baby ward were found to be infected with another of the 10 types of *Esch. coli* for which tests were being made. At some stage during their stay in hospital three (Cases 17, 20 and 24) were infected with O111 and two with O128 (Cases 5 and 14). One patient (Case 20) was found to be excreting O111 on admission and subsequently infected the other two; one of these was found to be excreting both O111 and O119 at the same time. The O128 was introduced by another infant who at no time excreted O119.

Other types of *Esch. coli* known to be associated with gastro-enteritis in infants were isolated from 12 other infants in the baby ward who were at no time found to be infected with O119. *Esch. coli* O128 was most common.

During the first interim period three of the five in-patients with O128 infection were in the baby ward and the organism appeared to have spread from one to another.

In the second epidemic none of the infants who were infected with O119 were found to have any other typable *Esch. coli* in their faeces. Three other infants in the baby ward and 11 others were found to be excreting one or other of the 10 types as shown in Table 4. The O26 which infected two infants in the baby ward appeared to have spread from one infant to the other.

In the second interim period the three in-patients infected with O55 were in the baby ward and again the infection appeared to spread from one to the other. This organism persisted in the ward into the third epidemic period and infected two of those who later excreted O119.

Evidence of Cross-infection. There were 23 infants infected with O119 during the main part of the first epidemic. Therefore the organism must have passed from one child to another at least 22 times; there was also evidence of cross-infection twice with O111 and once with O128. That is at least 25 incidents of cross-infection among 51 infants in 16 weeks. During the second epidemic there were 13 cross-infections due to O119 and one due to O26 among 47 infants in 11 weeks. In the three weeks of the third epidemic there were 18 at risk and three incidents of cross-infection. In the first interim period there were two examples due to O128 and in the second two due to O55. However, one other pathogenic type of *Esch. coli* was found in the ward during the second epidemic and three others during the second interim period without any spread.

Totalling up the number of days each child was at risk in the ward, there were 1,175 child-days in the first epidemic, 818 in the second and 231 in the third. Therefore there was one incident of cross-infection for every 47, 58 and 77 child-days in the three epidemics respectively.

These facts show a decreasing incidence of cross-infection. This could be due either to preventive measures or to decreasing virulence and infectivity of the organism. Table 1 does not show any decline in the incidence of symptoms in the later epidemics nor do the mortality figures suggest any decrease in the virulence of the organism as the type of infants at risk were very similar throughout the whole period. Therefore it was felt that the improved control of the infections was due to the measures taken to cut down cross-infection.

Improvements to Reduce Cross-infection. The general precautions against infectious diseases in force at the time of the first epidemic have been described earlier. During the first interim period the following improvements were made: (1) ample paper hand towels were provided; (2) taps were adjusted to be easily operated by elbow; (3) cello-

TABLE 4
NUMBERS OF INFANTS INFECTED WITH VARIOUS *ESCH. COLI* TYPES

Esch. coli Type	1st O119 Epidemic Sept. 1954-Jan. 1955		Interval		2nd O119 Epidemic June-Aug. 1955		Interval		3rd O119 Epidemic Feb. 1956	
	I.P.*	O.P.†	I.P.	O.P.	I.P.	O.P.	I.P.	O.P.	I.P.	O.P.
O119	28	1	0	0	15	5	1	7	4	0
O128	3	3	5	2	0	2	1	2	0	0
O55	1	2	2	1	2	0	3	2	2	0
O26	3	0	0	1	2	1	1	1	0	0
O111	3	0	0	0	0	1	2	2	0	0
O126	0	1	0	0	0	4	0	0	0	0
O86	0	1	0	0	2	0	0	0	0	0
O125	1	0	0	0	0	0	0	0	0	0
O114	0	0	0	0	0	0	1	0	0	0

* I.P. = In-patient in any ward of the hospital.
† O.P. = Out-patient.

phane was incorporated in face masks; (4) nurses responsible for making feeds no longer had nursing duties in the ward, leaving one sister, two staff nurses and eight student nurses to look after 12 babies. This staff was no longer called upon to help at night in Out-patients or in emergencies in other wards, but worked only in the baby ward.

The major structural improvements could not be carried out till August and September, 1955, after the second epidemic. Then the sluice room was redesigned and unnecessary equipment removed. A large bucket sterilizer and an electric incinerator were installed. Once the former was working, a special bucket and lid for soiled clothes, linens and napkins was provided for each baby. In the sluice room the nurses, using large forceps, put the napkins into one large container and the soiled laundry into another, spraying Domitol into the containers before replacing the lids. The napkins went in the inner bags of the container direct to the laundry. The soiled linen container was emptied down a shute four times a day by the porters. All the linen was then sorted and dispatched to the laundry by separate external staff from a special room in the basement.

A modern stainless steel kitchen sink unit was installed and each baby was given an individual feed tray on which was put his own feed box and lid for bottle and individual teat bowl and lid. All were sterilized before each feed and kept on a separate shelf in a cupboard.

Special precautions against infection were instituted whenever a baby passed suspicious motions. These consisted in using soluble napkins which were burnt in the electric incinerator, soaking all linen in Domitol in a separate container kept on the balcony outside the sluice, providing separate examination trays containing an auriscope, stethoscope, spatulae and torch, and keeping feeds in a separate kitchen. In addition all infants with symptoms of gastro-enteritis were treated with antibiotics, terramycin being usually chosen.

The effectiveness of these precautions has been further tested since the end of the period of this study when babies with gastro-enteritis due to *Esch. coli* O127 were admitted from the Maternity Unit. Although several babies became cross-infected, control was easy and no persistent epidemic occurred.

Discussion

Bacteriology. The association of certain types of *Esch. coli* with gastro-enteritis in infants is now well established. The subject has been thoroughly reviewed by Smith (1955). While some cases of gastro-enteritis may be caused by viruses or some yet unidentified agent there is no doubt in our minds that other cases are not only associated with the presence of certain *Esch. coli* types in the intestine but are actually caused by these organisms.

The first attempts to classify *Esch. coli* isolated from cases of gastro-enteritis used biochemical criteria but this method was insufficiently precise to definitely distinguish pathogenic from non-pathogenic strains. The serological classification of *Esch. coli* by Kauffmann (1947) based on O, K and H antigens allowed a much more exact identification. By the end of 1953 there were already 126 O groups, 62 K antigens and 34 H antigens recognized (Kauffmann, 1954). In *Esch. coli* isolated in gastro-enteritis the K antigens are of the B type, except in O25 strains in which they are of L type, and a constant B antigen appears to be associated with each O group. Each OB type may have variable H (flagellar) antigens or none at all. Variation in H antigen, then, allows subdivision of OB types which is useful in epidemiological studies.

Numerous papers incriminating at least 12 different O types of *Esch. coli* as causes of gastro-enteritis have been published. Kauffmann (1947) showed that strains collected from gastro-enteritis cases by Adam as early as 1923 and 1927 were type O111:B4 while those isolated by Bray (1945) were type O55:B5. Types O26:B6, O86:B7, O125:B15, O126:B16 and O128:B12 were found later by Taylor and Charter (1952) in infants with gastro-enteritis. More recently cases due to type O127:B8 have been described by Cooper, Walters, Keller, Sutherland and Wiseman (1955) and due to O114 by Rogers and Cracknell (1956) and Charter (1956).

Esch. coli later shown to be type O119:B14 were first isolated from calves with 'scours' (Wramby, 1948). Scours is a disease of calves in which fever, diarrhoea and pneumonia occur in the first few days after birth. Smith (1953) reported the association of this organism with diarrhoea in 21 infants in Aberdeen during 1952. Thomas and Charter (1956) record the isolation of O119 from one infant but no other paper describing this organism as a cause of epidemics of gastro-enteritis is known to us though a number of outbreaks have occurred (Smith, 1955).

The biochemical reactions of the Aberdeen strains, where tested, were similar to those of the strains isolated by us in Westminster and the strains in both epidemics were type H6 which is the most commonly found flagellar antigen in O119 strains. The virulence of the Aberdeen O119 organisms was comparatively like that of our strains. No deaths occurred in the 21 Aberdeen cases while three deaths

occurred in our series of 61 cases and two of those who died had other severe disabilities. In both series the infectivity rate was high. Smith (1953) states that 10 out of 25 contacts became infected. In our first epidemic 45%, in our second 30% and in our third 22% of the infants in the ward exposed to infection were found to be excreting the organism.

The reasons for incriminating *Esch. coli* O119 as the cause of gastro-enteritis in our cases were as follows: (*a*) out of the 20 cases of definite gastro-enteritis in the three epidemics, 12 developed symptoms within two days of the first demonstration of *Bact. coli* O119; (*b*) all had almost pure cultures of the organism from their faeces at the time that they were ill. In convalescence, however, the organism usually became gradually less predominant; (*c*) there was a parallelism in the ward between the incidence of positive swabs and cases of gastro-enteritis (Figs. 2 and 4); (*d*) *Esch. coli* O119 was cultured from all cases of gastro-enteritis in the ward during the epidemics and no other causal organism was found.

In contrast was our finding of negative faecal swabs not only in hundreds of babies without diarrhoea at this hospital, but also in more than 800 swabs from infants in the maternity units mentioned earlier.

Prevention of Cross-infection. The improvements in construction, nursing care and drug treatment in the baby ward, already described, undoubtedly reduced cross-infection but did not at once prevent fresh cases, even though stools were rapidly cleared of all *Esch. coli* (Figs. 1 and 3). This indicates the extreme infectivity of the pathogenic *Esch. coli* which together with the short incubation period allows epidemics to occur even in carefully managed baby wards and leaves no time for delay in taking extra precautions. This great infectivity has been commented on by many authors including Rogers (1951) and Jameson, Mann and Rothfield (1954).

A child excreting the organism, whether with or without symptoms, is a danger to others and should be sought out and isolated. Ideally this requires an isolation-admission ward where children who have been in contact with gastro-enteritis or recently in other hospitals or day nurseries can be swabbed and observed for at least two days. Jameson *et al.* (1954) not only recommended this but did not allow infants who had been previously in a gastro-enteritis ward to be admitted for any condition to a ward other than a gastro-enteritis unit. This was because such infants may be continuous or intermittent carriers of pathogenic *Esch. coli*. For the same reason they did not allow infants who had been in a gastro-enteritis unit to be transferred to nurseries or convalescent homes. Several of our infants continued to excrete O119 for about eight weeks and one infant with O111 infection was still excreting this organism after more than four months. We certainly agree that these infants should be nursed in isolation and we consider that excretors of pathogenic types of *Esch. coli* should be at once treated with larger than normal oral doses of antibiotics, such as terramycin, 50 mg./lb./day, or neomycin, 50 mg./lb./day. Sensitivity tests should be made on all strains isolated as there is considerable variation in sensitivity of these organisms to antibiotics and sulphonamides.

Summary

Three epidemics of gastro-enteritis due to *Esch. coli* O119 involved 45 babies in one ward. Twenty-seven had symptoms and three died.

The short incubation period and high infectivity rate allowed the occurrence of these epidemics in spite of usual barrier nursing techniques and individual cubicles. Cross-infection was reduced by improved precautions and prompt administration of terramycin to all babies excreting the organism. Sensitivity tests proved useful in the choice of antibiotic. All the O119 strains were resistant to sulphonamide and sensitive to chloramphenicol, aureomycin, streptomycin, neomycin and polymyxin.

In the 18 months' study 3,048 faecal specimens from 894 infants were examined. Apart from the 45 infants in the three *Esch. coli* O119 epidemics another 16 were found to be infected with O119 and 61 with other pathogenic types of *Esch. coli*, including 18 with O128, 15 with O55, nine with O26 and eight with O111.

We wish to thank Dr. I. M. Anderson, Dr. C. F. Harris, the Hon. Mrs. N. O. Richards, Mr. D. Levi and Mr. G. H. Macnab for their help and encouragement and their permission to report on their cases; Dr. Joan Taylor and Dr. J. Smith for much valuable advice and the supply of various typing antisera, and Miss Geraldine Coats for much hard work in performing the biochemical and sensitivity tests.

REFERENCES

Bray, J. (1945). *J. Path. Bact.*, **57**, 239.
Charter, R. E. (1956). *Ibid.*, **72**, 33.
Cooper, M. L., Walters, E. W., Keller, H. M., Sutherland, J. M. and Wiseman, H. J. (1955). *Pediatrics*, **16**, 215.
Jameson, J. E., Mann, T. P. and Rothfield, N. J. (1954). *Lancet*, **2**, 459.
Jewell, P. and Pearmain, G. E. G. (1954). *J. clin. Path.*, **7**, 308.
Kauffmann, F. (1947). *J. Immunol.*, **57**, 71.
—— (1954). *Enterobacteriaceae*, 2nd ed. Ejnar Munksgaard, Copenhagen.
Rogers, K. B. (1951). *Proc. roy. Soc. Med.*, **44**, 519.
—— and Cracknell, V. M. (1956). *J. Path. Bact.*, **72**, 27.
Smith, J. (1953). *Ibid.*, **66**, 503.
—— (1955). *The Aetiology of Epidemic Infantile Gastro-enteritis.* Royal College of Physicians, Edinburgh.
Taylor, J. and Charter, R. E. (1952). *J. Path. Bact.*, **64**, 715.
Thomas, M. E. M. and Charter, R. E. (1956). *Brit med. J.*, **2**, 339.
Topley, W. W. C. and Wilson, G. S. (1946). *Principles of Bacteriology and Immunity*, 3rd ed. Edward Arnold, London.
Wramby, G. (1948). *Acta path. microbiol. scand.*, Suppl. 76.

BOOK REVIEWS

The Medical Annual. Edited by SIR HENRY TIDY and Prof. R. MILNES WALKER. (Pp. xi + 580; 42 plates.) Bristol: John Wright. 1958.

The seventy-sixth issue of the *Medical Annual* will be the last to bear the name of Sir Henry Tidy as medical editor, and the publisher's note rightly deplores his departure and promises an appraisal of his work for the Annual next year.

Two major changes have been made. The alphabetical arrangement according to the title of the article had become clumsy and confusing, and a certain degree of grouping into sections has been introduced, which, the publishers maintain and the reviewer agrees, will make for ease of reference. The second innovation is a series of four special articles before the "Review of the year's work" namely, Intersexuality; Wound infection in operating theatres; Dangers arising from the medical use of x-rays; and Changing emphasis in paediatrics.

These articles are presumably intended as general reviews on chosen subjects of very particular interest and should they be greatly appreciated, and as a result increase in number, it will, to some extent, change the nature of the Annual. They are, as they should be, of a high standard though the last, dealing largely with the changes that have come over the practice of paediatrics as a result of the rapid decline of mortality and morbidity in children, the growth of psychiatry and the infiltration of a more psychological outlook in hospitals, is light-weight stuff compared at least with the first two.

The section on paediatrics seems, to a paediatrician, disproportionately short; nine medical and five surgical pages in a 570-page book leaves little enough room to review even the 14 subjects discussed though these have been usefully chosen. However, subjects of paediatric interest are scattered throughout the Annual ('paediatrics' we are constantly saying, 'is only general medicine for a special age group') and one can read about polio vaccination, the treatment of obesity with a high fat diet, metabolic hypothyroidism, coarctation of the aorta, Hirschsprung's disease and, in a particularly valuable article, rheumatoid arthritis. Here the discussion is of the relation of this condition to others such as disseminated lupus erythematosus and diffuse necrotizing polyarthritis, and of the dangers of hypercortinism, which may induce mesenchymal changes closely resembling either of these two conditions.

Finally, for light reading, there is an account ('instructive', says the Annual) in the section on the urogenital tract on the size of the male urethral meatus in the circumcised and uncircumcised. Apparently this is, on an average, smaller in circumcised adults, and the earlier circumcision is performed the smaller it is! Perhaps this stupendous bit of statistical research will initiate a swing against circumcision in the States similar to that which occurred in this country when the bottom dropped out of circumcision with the advent of the National Health Service.

The Annual ends with the Practitioner's Index and Book of the Year.

Enuresis: A Clinical and Genetic Study. By B. HALLGREN. (Pp. 159.) Copenhagen: E. Munksgaard. 1957.

This monograph contains a tremendous amount of factual material derived from a retrospective study of 111 enuretics treated at a psychiatric clinic in 1945 and a current study of 119 cases in 1952. On the clinical side the aspects studied included the history (85% primary, 15% acquired), sex differences, frequency, age of cessation, depth of sleep and association with emotional disturbances, speech disorders, masturbation, etc. Environmental factors reviewed included social grading, size of family and broken homes. The genetic/statistical section reveals how much work has been put into the study and how disappointingly indefinite have been the results. Many of the conclusions, which paediatricians experienced in the handling of such cases would accept without question, have not been statistically proven partly because of the ever present difficulty of separating heredity and environment and partly because enuretics form a heterogeneous group. Nevertheless, the author concludes that there is a group of genetically determined primary enuretics in whom the symptom may be suppressed by environmental harmony, and that the acquired cases may follow an environmental stimulus. He is on less sure ground when calculating the mode of inheritance but some evidence is given to show that the mechanism is one of simple dominance with incomplete penetrance, the latter probably being mainly influenced by external factors.

This monograph makes no claim to study treatment but a passing reference reveals that only 4·4% of the cases ceased to wet within six months after treatment, i.e. a therapeutic failure rate of 95·6%! It will be invaluable for anyone writing a paper on enuresis but almost valueless to anyone endeavouring to treat the individual case.

Kinderärztliche Notfallfibel, 4th ed. By B. de RUDDER. (Pp. viii + 205; 34 figures. DM 18.60.) Stuttgart: Georg Thieme. 1958.

The fourth edition of Professor de Rudder's by now well-known 'aide' to the diagnosis and treatment of medical and surgical emergencies in children has been somewhat expanded. While this increased covering of the subject must improve its value it is to be regretted that, from the point of view of the foreigner to German medicine, the table of drugs and their dosage is composed almost entirely of proprietary names which mean nothing to the reviewer.

INDEX TO VOLUME XXXIII

Abdominal pains, recurrent: field survey of 1,000 school children, 165
ABO incompatibility and haemolytic disease of newborn, 185
Abscess, acute spinal epidural, in infant with recovery, 261
Accra, intraperitoneal blood transfusion in treatment of kwashiorkor in children in, 202
Acidosis, renal, idiopathic, in infancy, prognosis of, 532
Acromegaly in a girl of 8 years, 49
Adenoma, parathyroid, in child presenting as rickets, 46
African children, phlyctenular conjunctivitis in, 292
ALLAN, J. H.: Mineralization of enamel in developing teeth, 182
Allergy to cow's milk in infancy, 265
Amino acids, plasma, in malnutrition, 499
Ammonia production in children, 532
Anaemia, haemolytic, acute acquired, associated with herpes simplex infection, 524
——, ——, associated with acute renal damage in infancy, 181
——, ——, in a child receiving nitrofurantoin, 527
——, ——, thrombotic microangiopathic, 520
Anorexia and severe inanition associated with tumour involving hypothalamus, 257
Antibodies which may be transferred to newborn baby, colostrum and milk as source of, 24
Antistreptolysin reaction in rheumatic children, 529
Aortic arch, congenital interruption of, 131
APLEY, J., and NAISH, N.: Recurrent abdominal pains; a field survey of 1,000 school children, 165
Arch, first, syndrome, 477
Archives of Disease in Childhood, on editing the, 383
ARMATA, J., *see* GIZA, T., and ARMATA, J.
Ataxia, Friedrich's, unusual findings in family with, 217
ATTA, S. B. G. O., *see* OFORI ATTA, S. B. G.
Autism, infantile, 382

B

Basophilic leucocytes in children in health and disease, 193
BENEŠ, V., *see* ELEFANT, E., VOJTA, V., and BENEŠ, V.
Beriberi in 4-month-old infant, 269
BEVERLEY, J.: Stress and pathogenicity of toxoplasma, 182
Bile ducts, intrahepatic, congenital absence of, 396
BISHTON, R. L., SAMES, C.P., and WALLIS, H. R. E.: Calcified mesenteric lymphangioma, 176
Blood transfusion, exchange, clinical and biochemical changes during, 142
—— ——, intraperitoneal, in children in Accra with special reference to treatment of kwashiorkor, 202
BOOK REVIEWS: BREMER, J. L.: Congenital anomalies of the viscera, 1957, 96; CHARNY, C. W., and WOLGIN, W.: Cryptorchism, 1957, 273; DANOWSKI, T. S.: Diabetes mellitus, 1957, 183; DAVISON, W. C., and LEVINTHAL, J. D.: The compleat pediatrician, 1958, 487; DELTHIL, P.: Les regimes du nourrisson bien portant ou malade, 1957, 274; DOUGLAS, J. W. B., and BLOMFIELD, J. M.: Children under five, 1958, 488; DUHAMEL, B., and SEGAUX, S.: Technique chirurgicale infantile, 1957, 487; DUHAMEL, J.: Affections non congénitales de l'anus et du rectum chez l'enfant, 1958, 384; EVANS, P., and MAC KEITH, R.: Infant feeding and feeding difficulties, 3rd edit., 1958, 274; FERGUSON, A. B., *et al.*: Orthopedic surgery in infancy and childhood, 1957, 272; GETZ, S. B., and REES, E. L.: The mentally ill child, 1957, 96; GUILLEMINET, M., and RICARD, R.: Pseudarthrose congénitale du tibia et son traitement, 1958, 384; HALLGREN, B.: Enuresis, 1957, 552; HINDEN, E.: A primer of paediatrics, 1957, 183; KAUFMANN, R.: Chirurgie préventive de la tuberculose ostéo-articulaire, 1958, 273; KJELLBERG, S. R., ERICSSON, N. O., and RUDHE, U.: The lower urinary tract in childhood, 1958, 274; KLEIN, M. R.: Hydrocéphalie du nourrison, 1958, 384; LIPPMAN, H. S.: Treatment of the child in emotional conflict, 1956, 275; LORAINE, J. A.: Clinical application of hormone assay, 1958, 274; Medical Annual, 1958, 552; MICHAUD, L., and DUCHÉ, D. J.: L'enfant inadapté, 1957, 276; MINSKI, L.: Deafness, mutism and mental deficiency in children, 1957, 275; MORLEY, M. E.: Cleft palate and speech, 4th edit., 1958, 276; New Zealand Commission of Inquiry. Fluoridation of public water supplies, 1957, 276; Paediatrics. Supplement, 1957, 272; ROBERTS, G. F.: Comparative aspects of haemolytic disease of the newborn, 1957, 384; RUDDER, B. de: Kinderärztliche Notfallfibel, 1958, 552; SCHAUFFLER, G. C.: Pediatric gynecology, 4th edit., 1958, 487; SPEER, F.: The management of childhood asthma, 1958, 487; VEEDER, B. S., *ed.*: Pediatric profiles, 1957, 272; WILKINS, L.: The diagnosis and treatment of endocrine disorders in childhood and adolescence, 2nd edit., 1957, 184; WISSLER, H.: Aktuelle Problem der Kindertuberkulose, 1958, 488; Year book of pediatrics, 1957-58, 272
BOORMAN, K. E., DODD, B. E., and GUNTHER, M.: A consideration of colostrum and milk as sources of antibodies which may be transferred to the newborn baby, 24
BOTHA, G. S. M.: The gastro-oesophageal region in infants, 78
Bowel negativism, implications of, in psychogenic megacolon in children, 371
BRETTELL, R. O., *see* GAIRDNER, D., MARKS, J., ROSCOE, J. D., and BRETTELL, R. O.
British Paediatric Association, 29th Annual General Meeting, 381
Bromide partition test in diagnosis of non-purulent meningitis, 440
Bronchiectasis in childhood, prognosis of, 19
BROWN, G. A., *see* WEEKS, M. M., and BROWN, G. A.
BUCHANAN, E. U., and KOMROWER, G. M.: The prognosis of idiopathic renal acidosis in infancy with observations on urine acidification and ammonia production in children, 532
BUCHANAN, M. F. G., *see* CRAIG, W. S., and BUCHANAN, M. F. G.
BULBROOK, R. D., GREENWOOD, F. C., and SNAITH, A. H.: Hormone excretion in precocious puberty in girls, 295
Burma, Upper, physical development of school girls in, 452
BURMAN, D.: Congenital spherocytosis in infancy, 335
BYWATERS, E. G. L., *see* HALLIDIE-SMITH, K. A., and BYWATERS, E. G. L.

C

Cardiovascular disease, congenital, during first year of life, electrocardiographic recognition of ventricular hypertrophy in, 346
Caries, dental, prevalence of, in relation to maturity, 455
CATHIE, I. A. B.: On editing the *Archives*, 383
CHEEK, D. B., and PERRY, J. W.: A salt wasting syndrome in infancy, 252
Chinese children, pneumatosis intestinalis, 445
CLAIREAUX, A. E., and FERREIRA, H. P.: Bilateral pulmonary agenesis, 364
COATES, S., *see* WOOLF, L. I., *et al.*
Coeliac disease, test for, 413
COGHLAN, M. K., and JOSEPH, M. C.: The disappearance of foetal haemoglobin in congenital cyanotic heart disease, 191
COLLINS, J.: Pancreatitis in young children, 432
Colon, neonatal perforation of, 362
Colostrum as source of antibodies which may be transferred to newborn baby, 24
Conjunctivitis, phlyctenular, in African children, 292
COOPER, C. E., and GRIFFIN, S.: Oesophageal stricture secondary to hiatus hernia, 383
CRAIG, W. S., and BUCHANAN, M. F. G.: Hypocalcaemic tetany developing within 36 hours of birth, 505
CRAVIOTO, J., *see* WESTALL, R. G., *et al.*
C-reactive protein determination in rheumatic children, 529
CROME, L., and SYLVESTER, P. E.: Hydranencephaly, 235
CROMPTON, C. W., *see* MAXWELL, G. M., ROWE, G. G., and CRUMPTON, C. W.
CROSSE, V. M., WALLIS, P. G., and WALSH, A. M.: Replacement transfusion as a means of preventing kernikterus of prematurity, 403
Cushing's syndrome in infant, 540

D

DAVIES, W.: Cow's milk allergy in infancy, 265
Deafness following maternal rubella; results of a prospective survey, 383
Dental caries, prevalence of, in relation to maturity, 455
Dermoid cyst, intracranial, associated with Klippel-Feil deformity and recurrent meningitis, 222
Development, assessment of, dissociation as guide to, 118
—— in children of very low birth weight, 10
Diabetes insipidus following tuberculous meningitis, 315
—— mellitus in child showing features of Refsum's syndrome, 320
Diabetic and non-diabetic women, postnatal weight loss of babies born to, 323
Diarrhoea, infantile, jaundice and, 330
Diastematomyelia, 536
DILLISTONE, F., *see* WOOLF, L. I., *et al.*
Dissociation as guide to developmental assessment, 118
DOBBS, R. H., *see* HAAS, L., and DOBBS, R. H.
DODD, B. E., *see* BOORMAN, K. E., DODD, B. E., and GUNTHER, M.
DRILLIEN, C. M.: Growth and development in a group of children of very low birth weight, 10
——: A longitudinal study of the growth and development of prematurely and maturely born children, 417, 423
Dwarfism, pituitary, autopsy findings in case of, 469
Dysautonomia, familial, 465
Dysmaturity, 123

E

EDEN, A. R., *see* ROBINSON, M. J., ORCHARD, N.P., and EDEN, A. R.
EDWARDS, M. S., GRIFFITHS, L. L., and SWIFT, P. N.: Cellular immunity at birth, 512
Electrophoresis, paper, of serum proteins in children, 61
ELEFANT, E., VOJTA, V., and BENEŠ, V.: Intraspinal neuroblastoma in a newborn baby, 212
EMERY, J. L., JOWETT, G. H., and MACDONALD, M. S.: Postnatal changes in glomeruli in the kidney, 182
Emphysema, regional obstructive, in infancy, 97
Enamel, mineralization of, in developing teeth, 182
ENGLESON, G., *see* SJÖSTEDT, S., ENGLESON, G., and ROOTH, G.
Enuresis, persistent, treatment with the electric buzzer, 160
ERICSSON, N. O., and IVEMARK, B. I.: Renal dysplasia and pyelonephritis in infants and children, 181
Erythema nodosum in children, changing aetiology of, 137
Erythrocyte sedimentation rate in rheumatic children, 529
Esch. coli O119 infection, epidemic of gastro-enteritis associated with, 543

F

FALKNER, F.: Some physical measurements in the first three years of life, 1
FARQUHAR, J. W., and SKLAROFF, S. A.: The postnatal weight loss of babies born to diabetic and non-diabetic women, 323
——, and SMITH, H.: Clinical and biochemical changes during exchange transfusion, 142
FAWNS, H. T., *see* NICOLS, V. S., and FAWNS, H. T.
Femur, traumatic subperiosteal haematoma of, in newborn, 205
FERREIRA, H. P., *see* CLAIREAUX, A. E., and FERREIRA, H. P.
Fibrinogen level in rheumatic children, 529
Fibrocystic disease, sweat analysis in, 74
FINDLAY, M., *see* GRIFFITHS, S. B., and FINDLAY, M.
First arch syndrome, 477
FISCH, L., *see* JACKSON, A. D. M., and FISCH, L.
Fluid shift from vascular compartment immediately after birth, 489
FOLEY, B. V.: Congenital interruption of aortic arch, 131
FOLL, C. V.: Physical development of schoolgirls in Upper Burma, 452
FORFAR, J. O., *see* THOMSON, J., and FORFAR, J. O.
FORSYTH, C. C., *see* PAYNE, W. W., and FORSYTH, C. C.
FRANKLIN, A. W.: The prognosis of bronchiectasis in childhood, 19
FREIMAN, I., *see* STEIN, H., and FREIMAN, I.
Friedrich's ataxia, unusual findings in family with, 217

G

GAIRDNER, D., MARKS, J., ROSCOE, J. D., and BRETTELL, R. O.: The fluid shift from the vascular compartment immediately after birth, 489
GAISFORD, W.: Poliomyelitis vaccination of newborn infants, 383
GARDNER, R., and SNAITH, A. H.: The urinary excretion of 17-hydroxysteroids in children, 305
Gargoylism; clinical, radiological and haematological features in two siblings, 229
Gastro-enteritis associated with *Esch. coli* O119 infection, epidemic of, 543
Gastro-oesophageal region in infants, anatomy of, with special reference to closing mechanism and partial thoracic stomach, 78
GEORGE, W. H., *see* WOOD, B. S. B., GEORGE, W. H., and ROBINSON, A. W.
GIZA, T., and ARMATA, J.: Frank beriberi in a 4-month-old infant, 269

Glucuronide formation, insufficient, in newborn and its relationship to pathogenesis of icterus neonatorum, 473
GOLDBLATT, E., and SNAITH, A. H.: Case of Cushing's syndrome in an infant, 540
GOMEZ, F., *see* WESTALL, R. G., *et al.*
GREENWOOD, F. C., *see* BULBROOK, R. D., GREENWOOD, F. C., and SNAITH, A. H.
GRIFFIN, S., *see* COOPER, C. E., and GRIFFIN, S.
GRIFFITHS, L. L., *see* EDWARDS, M. S., GRIFFITHS, L. L., and SWIFT, P. N.
GRIFFITHS, R., *see* WOOLF, L. I., *et al.*
GRIFFITHS, S. B., and FINDLAY, M.: Gargoylism: clinical, radiological and haematological features in two siblings, 229
Growth and development of prematurely and maturely born children, longitudinal study of, 417, 423
—— in children of very low birth weight, 10
Gumma of liver in congenital syphilis, 58
GUNTHER, M., *see* BOORMAN, K. E., DODD, B. E., and GUNTHER, M.
——, *see also* HASSAN, F., and GUNTHER, M.

H

HAAS, L.: Neonatal perforation of colon, 362
——, and DOBBS, R. H.: Congenital absence of the intrahepatic bile ducts, 396
Haemangioma, subglottic: two infants with laryngeal stridor, 210
Haematoma, traumatic subperiosteal, of femur in newborn, 205
Haemoglobin, foetal, disappearance of, in congenital cyanotic heart disease, 191
Haemolytic disease of newborn, ABO incompatibility and, 185
HALLIDIE-SMITH, K. A., and BYWATERS, E. G. L.: The differential diagnosis of rheumatic fever, 350
Hartnup disease, case of, 114
HASSAN, F. and GUNTHER, M. The serum proteins at 6 months of age in infants fed on human or cow's milk or on both, 30
Head, first arch syndrome, 477
Heart, auricular-ventricular dissociation, and Wenckebach phenomenon in children, 236
—— disease, congenital cyanotic, disappearance of foetal haemoglobin in, 191
——, ——, ——, in one of identical twins, 342
——, normal diameter of, in radiographs of pre-school children, 134
——, ventricular hypertrophy in congenital cardiovascular disease during first year of life, electrocardiographic recognition of, 346
——, —— septal defect in infants, clinical and haemodynamic study of, 67
Heberden's cases of anaphylactoid purpura, 271
HENDERSON, W.: A case of Hartnup disease, 114
Hernia, hiatus, oesophageal stricture secondary to, 383
Herpes simplex infection, acute acquired haemolytic anaemia associated with, 524
—— zoster ophthalmicus in children, 437
HEWITT, D.: Normal diameter of the heart in radiographs of pre-school children, 134
HEYCOCK, J. B., and WILSON, J.: Diabetes mellitus in a child showing features of Refsum's syndrome, 320
Hiatus in infants, 82
HOLBOROW, C. A.: Subglottic haemangioma, 210
HOLDEN, H. M., *see* MISCH, K. A., and HOLDEN, H. M.
HOLT, L. E., jun., *see* WESTALL, R. G., *et al.*
Hormone excretion in precocious puberty in girls, 295
HUGH-JONES, K., and ROSS, G. I. M.: Epidemics of gastro-enteritis associated with *Esch. coli* O119 infection, 543
HUSAIN, O. A. N., *see* WELCH, R. G., and HUSAIN, O. A. N.
Hydranencephaly, 235
Hydrocephalus, natural history of, 383
α-hydroxy-butyric acid and phenylpyruvic acid, inborn error of metabolism with urinary excretion of, 109
Hypercalcaemia, infantile, severe, with special reference to the facies, 385
Hypertension, essential, changes in placenta, in, 182
Hypocalcaemic tetany developing within 36 hours of birth, 505
Hypothalamus, tumour involving, anorexia and severe inanition associated with, 257

I

Icterus neonatorum, insufficient glucuronide formation in newborn and its relationship to pathogenesis of, 473
ILLINGWORTH, R. S.: Dissociation as guide to developmental assessment, 118
Immunity, cellular, at birth, 512
Inanition, severe, and anorexia associated with tumour involving hypothalamus, 257
Infants, newborn, colostrum and milk as source of antibodies which may be transferred to, 24
——, ——, haemolytic disease of, ABO incompatibility and, 185
——, ——, hypocalcaemic tetany in, 505
——, ——, insufficient glucuronide formation in, and its relationship to pathogenesis of icterus neonatorum, 473
——, ——, intraspinal neuroblastoma in, 212
——, ——, meningitis in, small outbreak in maternity unit, 358
——, ——, perforation of colon in, 362
——, ——, poliomyelitis vaccination of, 383
——, ——, traumatic subperiosteal haematoma of femur in, 205
——, ——, urinary infection in, diagnosis of, 382
IVEMARK, B. I., *see* ERICSSON, N. C., and IVEMARK, B. I.

J

JACKSON, A. D. M., and FISCH, L.: Deafness following maternal rubella, 383
JAMES, C. C. M., and LASSMANN, L. P.: Diastematomyelia, 536
JAMES, U.: The diagnosis of urinary infection in the newborn, 382
Jaundice and infantile diarrhoea, 330
JENKINSON, V., *see* PAYNE, W. W., and JENKINSON, V.
JOLLY, H., and LAMONT, E. M.: Sirenomelia, 226
JONES, H. E.: Congenital heart disease in one of identical twins, 342
JOSEPH, M. C., and PARROTT, D.: Severe infantile hypercalcaemia with special reference to the facies, 385
——, *see also* COGHLAN, M. K., and JOSEPH, M. C.
JOWETT, G. H., *see* EMERY, J. L., JOWETT, G. H., and MACDONALD, M. S.

K

KAGAN, H.: Anorexia and severe inanition associated with a tumour involving the hypothalamus, 257
Kanner's syndrome, 328
Kernikterus of prematurity, replacement transfusion as means of preventing, 403
17-ketosteroids, urinary excretion of, in children, 301
Kidneys, acidosis, idiopathic, in infancy, prognosis of, and urine acidification and ammonia production, 532
——, acute damage of, in infancy, haemolytic anaemia associated with, 181
——, dysplasia of, and pyelonephritis in infants and children, 181
——, glomeruli of, postnatal changes in, 182

Klippel-Feil deformity and recurrent meningitis, intracranial dermoid cyst associated with, 222
KOMROWER, G. M., *see* BUCHANAN, E. U., and KOMROWER, G. M.
Kwashiorkor, intraperitoneal blood transfusion in treatment of, in children in Accra, 202

L

LA PEÑA, C. DE, *see* WESTALL, R. G., *et al.*
LAMONT, E. M., *see* JOLLY, H., and LAMONT, E. M.
LASSMAN, L. P., *see* JAMES, C. C. M., and LASSMAN, L. P.
LAURENCE, K. M.: The natural history of hydrocephalus, 383
Leucocytes, basophilic, in children in health and disease, 193
LEVY, D., and PORGESS, A.: Haemolytic anaemia in child receiving nitrofurantoin, 527
Liver, gumma of, in congenital syphilis, 58
LORBER, J.: Changing aetiology of erythema nodosum in children, 137
——: Diabetes insipidus following tuberculous meningitis, 315
Lungs, agenesis, bilateral, 365
——, chronic disease of, sweat analysis in, 74
Lymphangioma, mesenteric, calcified, 176

M

MACDONALD, M. S., *see* EMERY, J. L., JOWETT, G. H., and MACDONALD, M. S.
MACFARLANE, A., and MACGREGOR, A. R.: Malignant non-chromaffin paraganglioma of thigh, 55
MACFARLANE, P. S.: Haemolytic anaemia associated with acute renal damage in infancy, 181
MACFARLANE, W. V., *see* WHITEHOUSE, D., and MACFARLANE, W. V.
MACGREGOR, A. R., *see* MACFARLANE, A., and MACGREGOR, A. R.
MACGREGOR, M.: Osteomyelitis variolosa, 383
——: Paediatrics in Western Nigeria, 277
MCKAY, E., and SMITH, J.: A small outbreak of neonatal meningitis in a maternity unit, 358
MCKENDRICK, T.: Familial dysautonomia, 465
MCKENZIE, J.: The first arch syndrome, 477
Malnutrition, plasma amino acids in, 499
MANSBRIDGE, J. N.: The prevalence of dental caries in relation to maturity, 455
MARKS, J., *see* GAIRDNER, D., MARKS, J., ROSCOE, J. D., and BRETTELL, R. O.
MARTIN, P. B., *see* RUSHWORTH, R. G., and MARTIN, P.B.
MAXWELL, G. M., ROWE, G. G., and CRUMPTON, C. W.: A clinical and haemodynamic study of ventricular septal defect in infants, 67
Measurements, physical, in first three years of life, 1
Megacolon, psychogenic, in children: implications of bowel negativism, 371
Meningitis, neonatal, small outbreak of, in a maternity unit, 358
——, non-purulent, bromide partition test in diagnosis of, 440
——, recurrent, and Klippel-Feil deformity, intracranial dermoid cyst associated with, 222
——, Salmonella, 171
——, tuberculous, diabetes insipidus following, 315
"Mermaid", 226
Mesenteric lymphangioma, calcified, 176
Metabolism, inborn error of, with urinary excretion of α-hydroxy-butyric acid and phenylpyruvic acid, 109
Milk as source of antibodies which may be transferred to newborn baby, 24
——, cow's, allergy in infancy, 265
——, human or cow's, or on both, serum proteins at 6 months of age in infants fed on, 30
MISCH, K. A., and HOLDEN, H. M.: Sweat test for the diagnosis of fibrocystic disease of pancreas, 179
MITCHELL, R. G.: Basophilic leucocytes in children in health and disease, 193
——, and RHANEY, K.: Traumatic subperiosteal haematoma of the femur in the newborn, 205
MONCRIEFF, A., *see* WOOLF, L. I., *et al.*
MUIR, C. S.: Pneumatosis intestinalis in Chinese children, 445
MULLINS, A. G.: The prognosis in juvenile obesity, 307

N

NAISH, N., *see* APLEY, J., and NAISH, N.
Neuroblastoma, intraspinal, in newborn baby, 212
Newborn infants, haemolytic disease of, ABO incompatibility and, 185
—— ——, hypocalcaemic tetany in, 505
—— ——, insufficient glucuronide formation in, and its relationship to pathogenesis of icterus neonatorum, 473
—— ——, intraspinal neuroblastoma in, 212
—— ——, meningitis in, small outbreak in maternity unit, 358
—— ——, milk and colostrum as source of antibodies which may be transferred to, 24
—— ——, perforation of colon in, 362
—— ——, poliomyelitis vaccination of, 383
—— ——, traumatic subperiosteal haematoma of femur in, 205
—— ——, urinary infection in, diagnosis of, 382
NICOL, V. S., and FAWNS, H. T.: Observations on the bromide partition test in the diagnosis of non-purulent meningitis, 440
Nigeria, Western, paediatrics in, 277
Nitrofurantoin, haemolytic anaemia in child receiving, 527

O

Obesity, juvenile, prognosis in, 307
O'DONOHOE, N. V., *see* TODD, R. MCL., and O'DONOHOE, N. V.
Oesophagus and stomach, closing mechanism between infants, 78
——, atresia of, with common tracheo-oesophageal tube, 367
——, congenital short, 89
——, stricture of, secondary to hiatus hernia, 383
OFORI ATTA, S. B. G.: Intraperitoneal blood transfusion in children in Accra with special reference to the treatment of kwashiorkor, 202
OLDHAM, J. S.: The electrocardiographic recognition of ventricular hypertrophy in congenital cardiovascular disease during the first year of life, 346
ORCHARD, N. P., *see* ROBINSON, M. J., ORCHARD, N. P., and EDEN, A. R.
Osteomyelitis variolosa, 383

P

Paediatric Pathology Club, Proceedings of 3rd Annual Meeting, 181
—— societies in British Isles, 95
Paediatrics in Western Nigeria, 277
PAINE, C. G.: Changes in the placenta with particular reference to essential hypertension and pre-eclamptic toxaemia, 182
Pancreas, fibrocystic disease of, sweat test for diagnosis of, 179
Pancreatitis in young children, 432
Paraganglioma, malignant non-chromaffin, of thigh, 55
Parathyroid adenoma in child presenting as rickets, 46
PARKER, R. G. F.: Jaundice and infantile diarrhoea, 330

PARROTT, D., *see* JOSEPH, M. C., and PARROTT, D.
PAYNE, W. W., and FORSYTH, C. C.: Paper electrophoresis of serum proteins in children, 61
——, and JENKINSON, V.: A test for coeliac disease, 413
PERRY, J. W., *see* CHEEK, D. B., and PERRY, J. W.
Phagocytic response, mechanism and nature of, 512
Phenylketonuria, dietary treatment of, 31
Phenylpyruvic acid and α-hydroxy-butyric acid, inborn error of metabolism with urinary excretion of, 109
Phreno-oesophageal membrane in infants, 83
PINKERTON, P.: Psychogenic megacolon in children: the implications of bowel negativism, 371
Pituitary dwarfism, autopsy findings in, 469
Placenta, changes in, with particular reference to essential hypertension and pre-eclamptic toxaemia, 182
Plasma amino acids in malnutrition, 499
Pneumatosis intestinalis in Chinese children, 445
Poliomyelitis vaccination of newborn infants, 383
POPOV, N., and STANISHEVA, S.: Erythrocyte sedimentation rate, C-reactive protein determination, serum protein fractions, fibrinogen level and anti-streptolysin reaction in 52 rheumatic children, 529
PORGESS, A., *see* LEVY, D., and PORGESS, A.
Prematurely born children, growth and development of, 417, 423
Prematurity, kernikterus of, replacement transfusion as means of preventing, 403
Proteins, serum, at 6 months of age in infants fed on human or cow's milk or both, 30
——, ——, in children, paper electrophoresis of, 61
PROUT, M., and SNAITH, A. H.: Urinary excretion of 17-ketosteroids in children, 301
Puberty, precocious, in girls, hormone excretion in, 295
PUGH, R. C. B.: Pyelonephritis, 181
Purpura, anaphylactoid, William Heberden's cases of, 271
Pyelonephritis, 181
——, renal dysplasia and, in infants and children, 181

R

RASMUSSEN, H., *see* WESTALL, R. G., *et al.*
Refsum's syndrome, diabetes mellitus in child showing features of, 320
RHANEY, K., *see* MITCHELL, R. G., and RHANEY, K.
Rheumatic children, erythrocyte sedimentation rate, C-reactive protein determination, serum protein fractions, fibrinogen level and anti-streptolysin reaction in, 529
—— fever, differential diagnosis, 350
Rickets, parathyroid adenoma in child presenting as, 46
ROBERTS, A. P.: Case of intracranial dermoid cyst associated with the Klippel-Feil deformity and recurrent meningitis, 222
ROBINSON, A. W., *see* WOOD, B. S. B., GEORGE, W. H., and ROBINSON, A. W.
ROBINSON, M. J., ORCHARD, N. P., and EDEN, A. R.: Thrombotic microangiopathic haemolytic anaemia, 520
ROITMAN, E., *see* WESTALL, R. G., *et al.*
ROOK, A.: William Heberden's cases of anaphylactoid purpura, 271
ROOTH, G., *see* SJÖSTEDT, S., ENGLESON, G., and ROOTH, G.
ROSCOE, J. D., *see* GAIRDNER, D., MARKS, J., ROSCOE, J. D., and BRETTELL, R. O.
ROSS, G. I. M., *see* HUGH-JONES, K., and ROSS, G. I. M.
ROSSI, M., *see* TOLENTINO, P., and ROSSI, M.
ROWE, G. G., *see* MAXWELL, G. M., ROWE, G. G., and CRUMPTON, C. W.
Rubella, maternal, deafness following: results of prospective survey, 383
RUSHWORTH, R. G., and MARTIN, P. B.: Acute spinal epidural abscess, 261

S

Salmonella meningitis, 171
Salt wasting syndrome in infancy, 252
SAMES, C. P., *see* BISHTON, R. L., SAMES, C. P., and WALLIS, H. R. E.
SANDISON, A. T.: Autopsy findings in a case of pituitary dwarfism, 469
Schoolgirls, physical development of, in Upper Burma, 452
Serum protein fractions in rheumatic children, 529
—— proteins at 6 months of age in infants fed on human or cow's milk or on both, 30
—— —— in children, paper electrophoresis of, 61
—— transaminase variations in childhood, 409
SHNIER, M. H.: The Wenckebach phenomenon and auricular-ventricular dissociation in children, 236
Sirenomelia, 226
SJÖSTEDT, S., ENGLESON, G., and ROOTH, G.: Dysmaturity, 123
SKLAROFF, S. A., *see* FARQUHAR, J. W., and SKLAROFF, S. A.
SMELLIE, J.: The effect on the infant of large therapeutic doses of vitamin D given to the mother throughout pregnancy, 383
SMITH, A. J., and STRANG, L. B.: An inborn error of metabolism with the urinary excretion of α-hydroxy-butyric acid and phenylpyruvic acid, 109
SMITH, H., *see* FARQUHAR, J. W., and SMITH, H.
SMITH, J., *see* MCKAY, E., and SMITH, J.
SNAITH, A. H., *see* BULBROOK, R. D., GREENWOOD, F. C., and SNAITH, A. H.
——, *see also* GARDNER, R., and SNAITH, A. H.
——, *see also* GOLDBLATT, E., and SNAITH, A. H.
——, *see also* PROUT, M., and SNAITH, A. H.
Spherocytosis, congenital, in infancy, 335
Spine, acute epidural abscess; case in infant with recovery, 261
——, neuroblastoma in, in newborn baby, 212
STANISHEVA, S., *see* POPOV, N., and STANISHEVA, S.
STEIN, H., and FREIMAN, I.: Phlyctenular conjunctivitis in African Children, 292
Stomach and oesophagus, closing mechanism between, in infants, 78
——, partial thoracic, in infants, 78
STONE, F. H.: Infantile autism, 328
STRANG, L. B., *see* SMITH, A. J., and STRANG, L. B.
Stridor, laryngeal, subglottic haemangioma in two infants, 210
Sweat analysis in fibrocystic disease, chronic pulmonary disease, and controls, 74
SWIFT, P. N., *see* EDWARDS, M. S., GRIFFITHS, L. L., and SWIFT, P. N.
SYLVESTER, P. E.: Some unusual findings in a family with Friedrich's ataxia, 217
——, *see also* CROME, L., and SYLVESTER, P. E.
Sympus dipus, 226
Syphilis, congenital, gumma of liver in, 58

T

Teeth, developing, mineralization of enamel in, 182
Tetany, hypocalcaemic, developing within 36 hours of birth, 505
Thigh, malignant non-chromaffin paraganglioma of, 55
THOMSON, J., and FORFAR, J. O.: Regional obstructive emphysema in infancy, 97
TODD, R. MCL.: Acromegaly in a girl of 8 years, 49
——, and O'DONOHOE, N. V.: Acute acquired haemolytic anaemia associated with herpes simplex infection, 524
TOLENTINO, P., and ROSSI, M.: Serum transaminase variations in childhood, 409

Toxaemia, pre-eclamptic, changes in placenta in, 182
Toxoplasma, stress and pathogenicity of, 182
Tracheo-oesophageal tube, common, in atresia of the oesophagus, 367
Transaminase, serum, variations in childhood, 409
Transfusion, exchange, clinical and biochemical changes during, 142
Tuberculous meningitis, diabetes insipidus following, 315
TUCKER, S. M.: Herpes zoster ophthalmicus in children, 437
Twins, identical, congenital heart disease in one of, 342

U

Urinary infection in newborn, diagnosis of, 382
Urine acidification in children, 532

V

VALENTINE, G. H.: ABO incompatibility and haemolytic disease of newborn, 185
Vascular compartment, fluid shift from, immediately after birth, 489
VEST, M.: Insufficient glucuronide formation in the newborn and its relationship to the pathogenesis of icterus neonatorum, 473
Vitamin D, large therapeutic doses of, given to mother during pregnancy, effect on infant, 383
VOJTA, V., *see* ELEFANT, E., VOJTA, V., and BENEŠ, V.

W

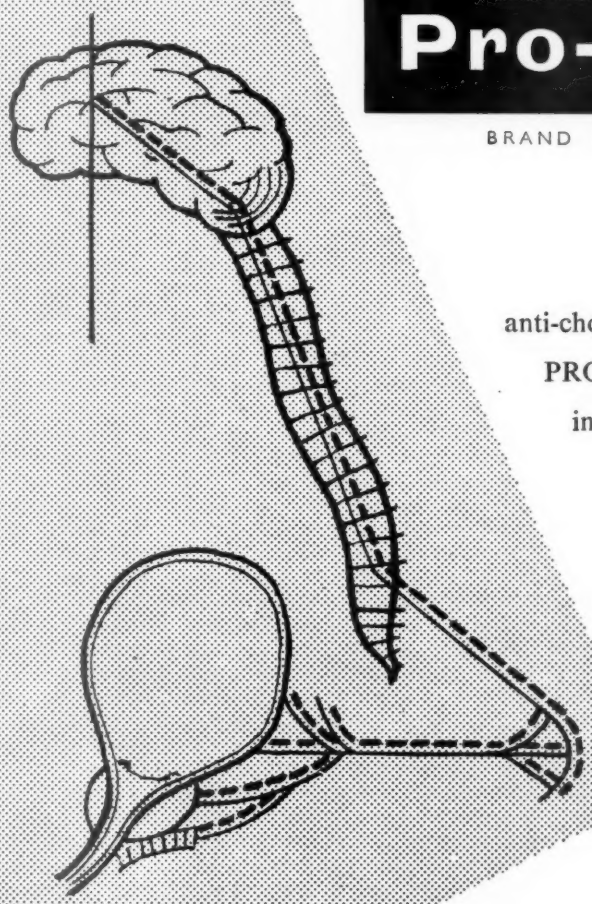
WALLIS, H. R. E., *see* BISHTON, R. L., SAMES, C. P., and WALLIS, H. R. E.
WALLIS, P. G., *see* CROSSE, V. M., WALLIS, P. G., and WALSH, A. M.
WALSH, A. M., *see* CROSSE, V. M., WALLIS, P. G., and WALSH, A. M.
WATSON, K. C.: Salmonella meningitis, 171
WEEKS, M. M., and BROWN, G. A.: Sweat analysis in fibrocystic disease, chronic pulmonary disease and controls, 74
Weight loss, postnatal, of babies born to diabetic and non-diabetic women, 323
WELCH, R. G., and HUSAIN, O. A. N.: Atresia of the oesophagus with common tracheo-oesophageal tube, 367
Wenckebach phenomenon and auricular-ventricular dissociation in children, 236
WESTALL, R. G., *et al.*: The plasma amino acids in malnutrition: preliminary observations, 499
WHITEHOUSE, D., and MACFARLANE, W. V.: Gumma of the liver in congenital syphilis, 58
WICKES, I. G.: Treatment of persistent enuresis with the electric buzzer, 160
WILSON, J., *see* HEYCOCK, J. B., and WILSON, J.
WOOD, B. S. B., GEORGE, W. H., and ROBINSON, A. W.: Parathyroid adenoma in a child presenting as rickets, 46
WOOLF, L. I., *et al.*: The dietary treatment of phenylketonuria, 31

PRINTED IN GREAT BRITAIN BY HEADLEY BROTHERS LTD
109 KINGSWAY LONDON WC2 AND ASHFORD KENT

In the treatment of Enuresis

Pro-Banthine

BRAND OF PROPANTHELINE BROMIDE



Well-established as the accepted oral anti-cholinergic agent for peptic ulcer symptoms, PRO-BANTHĪNE is also successfully used in the control of enuresis in cases which can be helped by inhibiting contraction of the bladder and thereby improving its capacity.

Both PRO-BANTHĪNE (15 mg.) and PRO-BANTHĪNE with Phenobarbitone, B.P., (15 mg. of each) are available as sugar-coated tablets in bottles of 40, 100 and 1,000. PRO-BANTHĪNE injections (30 mg.) are available in 1 c.c. vials, in boxes of 6 and 25.

Literature on request.

SEARLE

HIGH WYCOMBE, BUCKS.

You have good reason to prescribe Carnation Milk

CARNATION solves mother's problems in feed preparation—as well as meeting your exacting requirements. Clinical studies have proved that babies thrive on Carnation.

You can prescribe Carnation with complete confidence for the following reasons:—

DIGESTIBILITY Carnation Milk ensures maximum digestibility because of soft granular curds and homogenized butterfat.

STERILITY Carnation Milk is sterilized *after* the tins are sealed. No matter when or where Carnation is bought, its sterility is assured.

EASE OF PREPARATION Carnation Milk saves time in preparation of feeds because it blends *instantly* with boiled water.

FLEXIBILITY Carnation Milk feeds are easily adjusted to individual requirements.

ACCURACY Carnation Milk is a liquid and provides for accurate measurement of every feed.

VITAMIN D INCREASE Carnation Milk is adequately fortified to a safe level with Vitamin D.

UNIFORMITY Carnation Milk is uniform because every tin is processed to precise standards of butterfat and total solids.

These advantages uphold the highest standards of Infant Feeding when you prescribe Carnation.

"The Feeding of Infants"—a book specially prepared for doctors—together with reprints of clinical investigations and Carnation feeding charts are available from: The Medical Dept., General Milk Products Ltd., Bush House, Aldwych, London, W.C.2.

For the best start in life ... ***Carnation***

Announcing

SYRUP

Trade **'V-CIL-K'** Mark

PAEDIATRIC

This new paediatric syrup of 'V-Cil-K' gives even the youngest patient convenient and decisive penicillin therapy. Each 5 ml. contains 125 mg. penicillin V potassium—the most effective oral penicillin yet developed. Peak levels are attained within 30 minutes, from a syrup so pleasantly flavoured that regular dosage is assured.

The dose is ¼ to 1 teaspoonful four times daily.

Available in bottles to make 30 ml. and 60 ml., each with a 5 ml. plastic spoon.

SYRUP
'V-CIL-K'
PENICILLIN V POTASSIUM

Lilly

ELI LILLY AND COMPANY LIMITED · BASINGSTOKE · ENGLAND

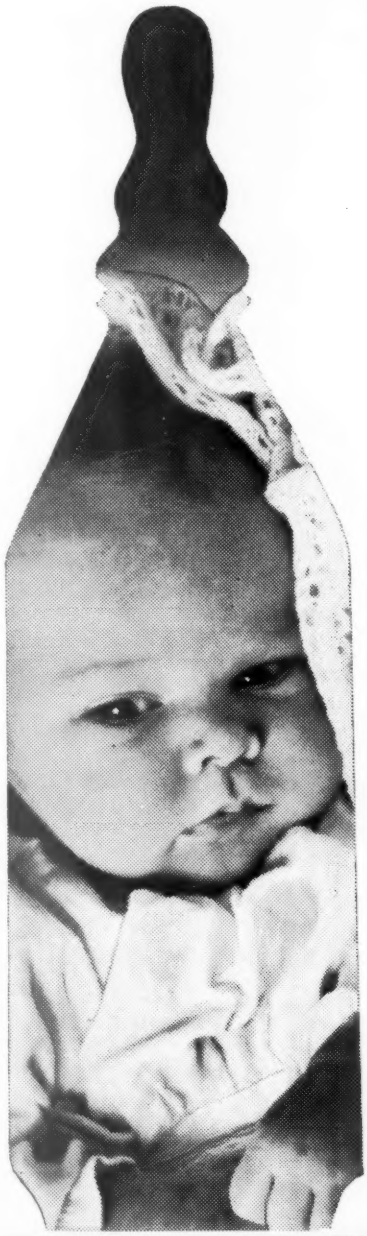
8

The excellent progress made by these lovely babies, the eighth set of Quads reared to date on Cow & Gate, again highlights the value of our wide range of specialized Infant Foods.
Anthony, David, Thelma and Beverley were started on Cow & Gate Frailac, progressed to Half Cream and are now thriving on Full Cream Cow & Gate supplemented by Cow & Gate Cereal Food.
For the abnormal birth, as for the normal baby, Cow & Gate Infant Foods still stand supreme.
A copy of our Medical Handbook giving a list of our products with their formulæ and dietary indications can be obtained on application to :-
The Medical and Research Department, Cow & Gate Ltd., Guildford, Surrey.

COW & GATE MILK FOODS

Guildford Surrey 5729

. . . **ABIDEC** DROPS



provide dependable vitamin protection

Balanced vitamin intake is provided with an easily administered single daily dose of Abidec Drops. Abidec Drops are miscible with milk and can be safely included in the feed without clogging the teat or separating out. This preparation provides vitamins A and D with C and members of the Vitamin B complex.

ABIDEC CAPSULES

administered in one daily dose of one capsule contain the same vitamins but in proportions designed for the requirements of older children and adults.

ABIDEC DROPS in bottles of 10 ml. and 50 ml. each with marked dropper.
ABIDEC CAPSULES in bottles of 30 and 250.

PARKE DAVIS & CO. LTD. (*Inc. U.S.A.*) HOUNSLOW, MIDDLESEX. TEL: HOUNSLOW 2361

1004

Now Ready

just published by

The Year Book Publishers, Inc., Chicago:

ADVANCES IN PEDIATRICS

VOLUME X. 362 pages, numerous illustrations and tables

Edited by **S. Z. LEVINE, M.D.,** *Cornell University Medical College, New York* **67s.**

CONTENTS: Psychologic Principles in Pediatric Practice: the Pediatrician and the Sick Child—*B. M. Korsch*; Treatment of Tuberculosis in Childhood—*E. M. Lincoln*; Convulsive Disorders in Infants and Children—*S. Livingston*; Prevention of Poliomyelitis by Vaccination—*A. B. Sabin*; Staphylococcal Infections in Nurseries—*T. E. Shaffer, J. N. Baldwin* and *W. E. Wheeler*; Muscular Disorders of Childhood—*F. H. Tyler*; The Transfer of Antibodies from Mother to Offspring—*B. Vahlquist*; Subject Index; Author Index.

Previous volumes available: **III** (1948) 60s.; **VI** (1953) 58s.; **VII** (1955) 62s.; **VIII** (1956) 62s.; **IX** (1957) 68s. *Volumes* **I, II, IV** *and* **V** *are out of print.*

This series of volumes provide new information, and chart future trends, in pediatric research; the reviews are comprehensive and scholarly. Both the specialist and general practitioner will discover in these books a wealth of timely information.

Distributed in the United Kingdom by

INTERSCIENCE PUBLISHERS, LTD.

88-90 Chancery Lane London, W.C.2

Now brought up to date by 1958 Supplement

PAEDIATRICS

FOR THE PRACTITIONER

Under the General Editorship of WILFRID GAISFORD, M.D., M.SC., F.R.C.P. and REGINALD LIGHTWOOD, M.D., F.R.C.P., D.P.H.

"These beautifully produced volumes are as much credit to the publishers as their contents are to the eminent team of paediatricians and others who have been co-opted from all over the world to comprise *Paediatrics for the Practitioner* . . . there is an almost complete coverage of paediatric subjects . . ."—*Archives of Disease in Childhood.*

In Three Volumes, Index Volume and 1956, 1957 and 1958 Supplements
Fully Illustrated. Price £14 10s. net per set, carriage and packing 7s. 6d. extra
1958 Supplement only, 35s. net, by post 1s. 6d. extra

Further details and Illustrated Prospectus on application

BUTTERWORTHS - 88 KINGSWAY, LONDON, W.C.2

Showroom: 11-12 BELL YARD, TEMPLE BAR, LONDON, W.C.2

Terramycin*

brand of oxytetracycline

—AN EXPANDING PATTERN OF USEFULNESS

Since the introduction of Terramycin a tremendous body of evidence has been published about this outstanding broad-spectrum antibiotic. Today, its value is known and established in almost every type of disease where infection is a factor. Following the course of this development we have introduced a wide variety of dosage forms, so that the clinical effectiveness of Terramycin should be enhanced by convenience and acceptability of presentation.

The very bulk of the available evidence becomes almost an embarrassment to the busy practioner who—having accepted the clinical value of Terramycin—wants a concise picture of indications, dosage, therapeutic effect, and possible side effects, in some specific condition. To overcome this problem we are preparing a series of brief monographs on the use of Terramycin in specific groups of indications. The first of these, 'Terramycin in the treatment of Bronchitis and Bronchiectasis', will be available shortly.

PFIZER PHARMACEUTICALS

Manufactured at Sandwich and processed at Folkestone, Kent

Pfizer

WHERE A BROAD-SPECTRUM ANTIBIOTIC IS INDICATED

Terramycin

Proved clinical effectiveness
Wide margin of safety
Complete range of dosage forms

**Registered Trade Mark*

067/P

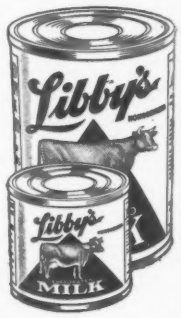
They both do well

on LIBBY'S milk

WHAT A FINE INFANT FOOD! Libby's is already safe and sterile as it comes from the tin. With Libby's, the child's formula is quickly made—and easily varied.

LIBBY'S contains all the goodness of fresh cow's milk. Yet the processing reduces curd particle size and—even more important—lowers curd tension. Thus Libby's closely resembles human milk, and is easy to digest from birth onwards.

AND WHAT A FINE FOOD FOR THE MOTHER! By using Libby's recipes, she can enjoy a varied diet that gives her the extra calcium that she needs. *Recipes, formulae and full information are in Libby's booklet, "Infant Feeding with Evaporated Milk". Write for your copy today.*



Libby's Full Cream Evaporated MILK

Libby, McNeill & Libby Ltd., Forum House, 15 & 16 Lime St. London, E.C.3.

THE NUFFIELD FOUNDATION

NUFFIELD INSTITUTE FOR MEDICAL RESEARCH

Research Fellowships

The Nuffield Foundation, as an experiment, is prepared to offer one research fellowship annually in each of the three years 1958, 1959 and 1960, tenable at the Nuffield Institute for Medical Research at Oxford, to enable a suitably qualified person who intends to follow an academic career in obstetrics or paediatrics in the United Kingdom to concentrate for a year on animal experiments directly related to basic physiology or pharmacology in the foetus or the new-born.

Each fellowship will be tenable for one year and is limited to a graduate in medicine of a University within the British Commonwealth, who should *either* have completed an appointment as Registrar (or its equivalent) in an approved department of obstetrics or paediatrics, *or* be seconded for one year from such an appointment. He should also have obtained the higher qualification appropriate to his chosen speciality. The stipend will be within the approved range of salaries for Senior Registrars under the National Health Service. Applications for the award beginning in 1959 must be received before 15th February, 1959 by the Director, The Nuffield Foundation, Nuffield Lodge, Regent's Park, N.W.1, from whom full particulars and application forms can be obtained.

L. FARRER-BROWN

Director of the Nuffield Foundation

ER, 195

E.C.3.

Vitamins in Prenatal Life

Vitamins are essential for the normal development of the embryo. Severe vitamin deficiencies during pregnancy, apart from their ill-effects on the mother, lead to death or malformation of the foetus. It is known from animal experiments that riboflavin is required in the later stages of bone development. Deformities which resemble human congenital malformations have also been observed following deficiencies of folic and pantothenic acids in the maternal diet. Although it is not possible to apply these results in detail to humans, adequate nutrition during pregnancy has long been considered to be of supreme importance.

Marmite is a useful source of every known factor of the vitamin B complex and is widely recommended to expectant mothers. Its appetising flavour ensures easy administration; it may be given as a drink made with boiling water or hot milk, or in sandwiches, or used in cooking.

MARMITE

yeast extract *contains* RIBOFLAVIN (*vitamin* B_2) 1.5 mg. per oz. NIACIN (*nicotinic acid*) 16.5 mg. per oz.

MARMITE LIMITED, 35 SEETHING LANE, LONDON, E.C.3

ADC 5608

ANNALES PAEDIATRICI

International Review of Pediatrics

(Published since 1858)

Selection of articles published recently:

Transposition des gros vaisseaux avec interruption de l'arc aortique, persistance du canal de Botal et communication interventriculaire. By *J. P. Christen, N. Genton* and *B. Volet*, Lausanne.

Convulsions in Childhood. By *Hans Zellweger*, Beirut.

Investigation of Bilirubin Tolerance of New-Born Rats in Anoxic Medium. By *Gy. Iványi* and *E. Dux*, Szeged.

Pathogenic Strain of Coli (O,111) among Prematures and the Use of Human Milk in Controlling the Outbreak of Diarrhea. By *S. Svirsky-Gross*, Tel-Aviv.

The Clinical Usefulness of the Diphenylamine Reaction in Pediatrics, with special Reference to Rheumatic Fever. By *M. Koltay, V. Endrei*, and *P. Ebrey*, Szeged.

Two volumes (of 6 issues each) are published every year. Price per volume: 95s.

S. KARGER, Basel, Switzerland

Agents for the United Kingdom:

INTERSCIENCE PUBLISHERS LTD., 88-90 CHANCERY LANE, LONDON, W.C.2

For the medical treatment of hypertrophic pyloric stenosis

SKOPYL BRAND OF METHYL SCOPOLAMINE NITRATE

a powerful antispasmodic without central depressant action

SKOPYL* is one of the most powerful spasmolytics known, the effect in vitro being five times greater than that of atropine, and ten times greater than that of scopolamine. The toxicity is three times that of scopolamine. Because of the greater activity the dose and the relative toxicity are correspondingly less.

SKOPYL has been in clinical use for about ten years. Comprehensive experience has shown the preparation to be especially valuable in all conditions where spasmolysis and inhibition of secretion is desirable. Side-effects are rare and usually very slight, even when large doses are used.

IT IS in paediatrics that SKOPYL is most useful, and the value of the preparation is best seen in the treatment of hypertrophic pyloric stenosis, where it has been proved able to replace surgery in many cases. Good results have also been obtained in infantile dyspepsia, and during the spasmodic period in pertussis.

For clinical references and directions for dosage, see a special booklet that will be sent on request.

SKOPYL is available in tablets of 0.5 mg. and 0.1 mg. (SKOPYL MITE), and in 0.25% w/v solution for sublingual use.

PHARMACIA Ltd.
UPPSALA, SWEDEN

represented by

SAVORY & MOORE, LTD.
61 Welbeck Street, London, W.1

*Trade Mark

9.17

PRINTED IN GREAT BRITAIN BY HEADLEY BROTHERS LTD 109 KINGSWAY LONDON WC2 AND ASHFORD KENT